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Horticultural potential of Nyssa biflora Walt. (swamp tupelo)

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DEDICATION

This thesis is dedicated to my family and friends.

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ABSTRACT

Nyssa biflora Walt. (swamp tupelo) and *Nyssa sylvatica* Marsh. (black gum) are closely related species of trees native to North America. Although *N. sylvatica* is prevalent in the nursery trade, little is known about the horticultural potential of *N. biflora*. Because many authors referred to *N. biflora* as a variant of *N. sylvatica*, taxonomic confusion may have resulted in the overlooking of *N. biflora* for use as a horticultural plant. The overarching objectives of my research were to establish seed-propagation protocols for *N. biflora* and to characterize the responses of seedlings of *N. biflora* to water stress. We included *N. sylvatica* in all experiments to provide a basis for comparison with *N. biflora.* The germination experiment was designed to test for effects of time of stratification on cleaned seeds (depulped seeds with endocarp intact) of *N. biflora* and *N. sylvatica;* seeds within intact fruits of *N. biflora* were also included. We found the pulp of fruit of *N. biflora* to have inhibitory effects on germination of seeds. Stratification had a more pronounced effect on *N. sylvatica* than *N. biflora,* and we conclude *N. biflora* is less resistant to germination. The water-stress experiment was designed to test for effects of a wide range of root-zone moisture contents on photosynthesis, water potential, and biomass accumulation of both species of *Nyssa*. Rates of photosynthesis of *N. biflora* receiving treatments of drought cycles were greater than those of *N. sylvatica* after two weeks, thus, *N. biflora* may be better adapted to short periods of drought than *N. sylvatica*. Rate of dry mass accumulation and increase in height of *N. biflora* were greater than those of *N. sylvatica* across all treatments of flooding and drought. *N. biflora* displayed a tolerance to flooded root zones that was superior to that of *N. sylvatica*. Adventitious roots and hypertrophied lenticels were formed on flooded plants of *N. biflora*. We conclude that *N. biflora* can be propagated easily from seed, is more resistant to water stress than is *N. sylvatica*, and deserves further investigation for its potential in horticulture.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Thesis Organization

This thesis includes four chapters. Chapter one is a literature review on the nomenclature of *N. biflora* and *N. sylvatica*, life history traits of *N. biflora*, propagation of *N. sylvatica*, germination inhibitors of fruit pulp, responses of plants to water stress, and responses of *N. biflora* and *N. sylvatica* to water stress. Following the review of literature, I include a chapter, in manuscript form, which was research conducted during my first year as a master's student. This manuscript has been submitted to *HortScience* for review. The third chapter is in manuscript form also, was a large part of my research conducted in the summer of 2014, and has been formatted to be submitted to *HortScience*. The final chapter includes a discussion of general conclusions and suggestions for future research. The appendices include images of adventitious roots and hypertrophied lenticels of *N. biflora* formed during the water-stress experiment, the protocol I used for measuring red-to-green stress index of leaves, and a description of a grafting experiment with *N. biflora* and *N. sylvatica*.

Literature Review

Nomenclature of *N. biflora* and *N. sylvatica*

Differences in ecophysiology or hybridization of species have been suggested reasons for resemblance between *N. biflora* and *N. sylvatica* (Burckhalter, 1992). *N. biflora* has been referred to as a wetland or moist lowland variety of *N. sylvatica* [*N. sylvatica* Marsh var. *biflora* (Walt.) Sarg.] (Eyde, 1963;; Godfrey and Wooten, 1981; Godfrey 1988; Little, 1971). *N. biflora* usually has oblanceolate leaf blades with entire margins, pistillate flowers occurring in pairs, and a buttressed trunk when growing in standing water (Gleason and Cronquist, 1991; Yatskievych, 2006). *N. sylvatica* usually has elliptic to obovate leaf blades (toothed margins occasionally), pistillate flowers mostly 2-5 per inflorescence, and

never develops a buttressed trunk. *N. biflora* also produces shorter fruiting peduncles than *N. sylvatica* (1-3 cm vs. 3-6 cm) (Gleason and Cronquist, 1991). The natural range of *N. sylvatica* is much more extensive than that of *N. biflora*, occurring from dry upland sites to moist bottomlands that are inundated occasionally (Burckhalter, 1990). *N. biflora* is restricted to inundated sites and occasionally in the margins of inundated sites; the habitats of the two species overlap rarely. When *N. sylvatica* occurs in lowland sites, the leaves can be narrower than leaves of nearby trees growing in upland sites and resemble leaves of *N. biflora* (Burckhalter, 1992). Contrarily, there have been populations discovered with no difference in leaf morphology between upland and lowland individuals of *N. sylvatica*. There has been some confusion with differentiating specimens of *N. biflora* and lowland *N. sylvatica* when specimens lacked flowers (Burckhalter, 1992). In a recent study of systematics of *Nyssa* of North America, flavonoid chemistry from all collections of *N. sylvatica* at bottomlands sites indicated that *N. biflora* and *N. sylvatica* are different species (Burckhalter, 1990). Burckhalter (1990) also concluded that *N. biflora* was more closely related to *Nyssa aquatica* L. (water tupelo) than to *N. sylvatica*. Below is Burckhalter's (1992) proposed key to the species of *Nyssa* of North America:

1. Trees; lowland to upland habitats; fruits in clusters usually 4 or 5..... *Nyssa sylvatica*
1. Trees or shrubs; very moist habitats, swamps, usually inundated; fruits 1 or 2 per peduncle 2
2. Leaves narrowly lanceolate, entire, usually 3 – 14 cm long; fruits usually 2 per peduncle; mature fruit blue-black, 0.6 – 1.4 cm long 3
2. Leaves widely ovate to obovate, often dentate toward apex, usually 8 – 25 cm long; fruits usually 1 per peduncle; mature fruit olive green to orange to light brown, 2 – 4 cm Long..... 4
3. Trees; leaves 5 – 14 cm long; fruit oblong, peduncle 1 – 4 cm long..... *Nyssa biflora*
3. Shrubs; leaves 3 – 6 cm long; fruit globose, peduncle 0.5 – 2 cm long ..... *Nyssa ursina*

4. Leaves widely obovate, often dentate toward apex, 10 – 25 cm long, petiole 2 – 5 cm long; mature fruit olive green to brown ..... *Nyssa aquatica*

4. Leaves narrowly to widely ovate, occasionally dentate throughout, 8 – 18 cm long, petiole 0.5 – 1.5 cm long; mature fruit dull yellow to orange..... *Nyssa ogeche*

Life history traits of *N. biflora*

N. biflora is a polygamo-dioecious trees species; trees can have staminate and perfect flowers or pistillate and perfect flowers (Schopmeyer, 1974). The flowers bloom in spring and are small, greenish-white (Radford et al., 1964); pollination is mainly completed by bees and also by wind (Schopmeyer, 1974). The fruit of *N. biflora* is a drupe that ripens in the fall and turns from green to dark blue when mature. Production of seeds of *N. biflora* varies from year to year, and late freezes may impair seed development. DeBell and Hook (1969) discovered that once seeds reached maturation, fruit drop occurred over a four-month period, but more than 50% of the seeds drop during November. By mid-December, 95% of the seed crop for that year had abscised from the trees. Seeds collected after the peak germination period (when exocarps are blue) had higher germination rates than seeds collected before the peak germination period (when exocarps are green) (DeBell and Hook, 1969). In addition to gravity, migratory robins (*Turdus migratorius* L.) play a major role in the dispersal of seeds of *N. biflora* (DeBell and Auld, 1968). Unlike fruits of *N. aquatica* (water tupelo), fruits of *N. biflora* sink when they fall in to the water.

Germination of seeds of *N. biflora* is epigeal (Schopmeyer, 1974) and occurs the spring following dissemination in the wild. Seeds will not germinate under water, indicating the water levels must subside before germination can commence (DeBell and Naylor, 1972). Newly emerged seedlings will grow quickly to ensure the apex and leaves of the plant remain above the water level because submergence during the growing season will result in death; submergence during the dormant season is not detrimental to plant health (Outcalt, 1990). *N. biflora* is considered not tolerant of shade and

seedlings germinated under shaded conditions will stay suppressed until exposed to sunlight (McGarity, 1979; Stubbs, 1973). In the wild, *N. biflora* can grow to heights of 37 meters and diameters of trunks can exceed one meter. Trees of *N. biflora* develop a buttressed trunk near the mean height of the water level. Under conditions of flooding, plants of *N. biflora* are capable of forming adventitious roots near water-level that are important to survival during periods of flooding (Hook et al., 1970a; Hook et al., 1971). *N. biflora* sprouts prolifically from suppressed buds when stumps are cut near the base (Hook and DeBell, 1970).

Propagation of *N. sylvatica*

Embryos of seeds of *N. sylvatica* exhibit moderate dormancies that can be overcome with cold, moist stratification for 60 to 90 days at 41° F (Dirr and Heuser, 2006). Dirr noted that the requirement of stratification can vary depending on seed provenance. To our knowledge, seed propagation protocols are nonexistent for *N. biflora* and may contribute to its lack of use as a landscape plant.

Although newer horticultural selections of *N. sylvatica* are budded or whip grafted onto seedling rootstock (Dirr and Heuser, 2006; Dirr, 2009), softwood stem cuttings of *N. sylvatica* can be rooted with little technical difficulty (Dirr, 2009). Softwood cuttings of *N. sylvatica* can be rooted by excising 3.8 cm shoots, followed by an application of 8,000 ppm IBA talc and stuck in a sand medium in a greenhouse at 70° F. It is recommended that the flat of cuttings receive bottom heat and then placed under mist; rooting will generally take place within two to three weeks. Newly rooted cuttings can be transplanted after six weeks and grown normally. High rooting percentages were observed following this protocol (95 to 100%) (Dummer, 1968). Propagation through methods of tissue culture have been successful as well (Brand and Lineberger, 1986; Kane et al., 1988). Although methods of asexual propagation of *N. sylvatica* have been investigated, we are unaware of any research conducted to propagate *N. biflora* by methods of asexual propagation.

Germination inhibitors of fruit pulp

Some authors have proposed that pulp of fruits can contain germination inhibitors (Bradbeer, 1988; Mayer and Polijakoff-Mayber, 1989). Greenberg et al. (2001) found that germination of seeds of *Celastrus orbiculatus* Thunb. (Oriental bittersweet) was delayed when seeds remained within intact fruits. Other studies have suggested that the juice from pulp of fruits contain germination inhibitors (Hayashida et al., 1994). Yagihashi et al. (1999) found that germination of seeds of two species of *Prunus* L. had germination inhibited by presence of fruit pulp; germination was also inhibited when seeds were treated with juice of fruit pulp. In a study evaluating the autotoxictiy of *Phytolacca americana* L. (poke weed), researchers found extracts from leaves, stems, and immature fruits capable of inhibiting germination of its own seeds (Edwards et al., 1988). Seeds of some species of plants cannot germinate unless ingested by birds (Noble, 1975; Temple, 1977). McEwan et al. (2010) studied germination of seeds of *Festuca arundinaceae* Schreb. (tall fescue), *Impatiens walleriana* Hook f. (impatiens), *Coreopsis lanceolata* L. (lanceleaf coreopsis), and *Poa pratensis* L. (Kentucky bluegrass) when treated with fruit extracts from *Lonicera maackii* (Rupr.) Maxim. (Amur honeysuckle) and *Lindera benzoin* L. Blume (spicebush). They found the fruit extract of *L. maackii* had an inhibitory effect on germination on seeds of all four species. Groot and Karssen (1992) found that seeds of ABA-deficient mutant tomatoes germinated readily and even within overripe fruits. Kobayashi et al. (2010) proposed that prevention of viviparous germination in *Citrullus lanatus* (Thunb.) Mansf. (watermelon) is due to accumulation of ABA in the flesh of fruits. Fruit pulp and exocarps with relatively thick cuticular layers can inhibit germination by their high osmotic pressure from dissolved sugars and reduced permeability of water which are factors that affect water relations of seeds, thus, affecting germination (Mayer and Poljakoff-Mayber, 1989).

Responses of plants to water stress

Hydrologic conditions of wetland environments can range from soil saturation to permanent inundation (Tiner, 1991). In wetlands where stagnant water is present, oxygen cannot diffuse efficiently into the water to compensate for root respiration, resulting in anaerobic soils (Brix and Sorrel, 1996). Less oxygen will typically become available deeper in the soil profile.

Plants exposed to flooding can have photosynthesis, respiration, and water and nutrient relations greatly affected. Photosynthesis is affected by decrease in stomatal conductance, this occurs relatively quickly on the onset of flooding (Pienkowski et al., 1998; Pezeshki, 2001). Reduction in stomatal conductance is triggered by decreasing cytokinin synthesis and increasing ABA (Pereira and Kozlowski, 1977). When flooding with salt water occurs, it creates an osmotic drought for plants that are sensitive. Morphological changes to plants in response to flooding include development of hypertrophied lenticels, adventitious roots, buttressed trunks, pneumatophores, and formation of aerenchyma tissue. Flooding can cause increased production of ethylene by stems, which is associated with development of hypertrophied lenticels. These lenticels aid in increasing the transport of gases from the atmosphere to tissues of the plant (Hook et al., 1970a).

Adventitious roots form at the water level and grow into the surface sediments to optimize on hypoxic conditions (Visser et al., 2000). Although shallow roots are advantageous to surviving anoxic conditions, the plant may become susceptible to mechanical stress. Rapidly moving water can cause damage by uprooting individuals (Schutten et al., 2005). Buttresses alleviate the problem of uprooting and contribute to the anchorage of a tree more than thin laterals of non-buttressed trees (Crook et al., 1997). In species such as *Taxodium distichum* L. (bald cypress), pneumatophores or “knees” are produced in relation to the depth of flood waters (Yamamoto, 1992). Pneumatophores are vertical root extensions that allow an oxygen pathway to roots to be formed (Kramer et al., 1953). Pneumatophores

can have specialized tissue termed aerenchyma (Purnobasuki and Suzuki, 2004; Purnobasuki and Suzuki, 2005). This tissue is characterized by its spongy nature with large intercellular air spaces in leaves, stems, roots which aid in circulation of gases. Aerenchyma tissue in woody plants has a major role of increasing porosity for oxygen transport from leaves to roots, as well as other gases from the roots to leaves. By having aerenchyma tissues, oxygen can leak out of the roots with less resistance and oxidize the rhizosphere. As this happens, the plants create an oxidized environment around the roots which can increase the obtainability of oxidized nutrients. The increase in nutrient availability is due to the aerobic microbes that are capable of surviving in the oxidized rhizosphere (Jackson and Armstrong, 1999; Maricle and Lee, 2002; Smirnoff and Crawford, 1983). Wetland plants often reduce their root : shoot while experiencing flooding (Smirnoff and Crawford, 1983). The reduction in root systems coupled with increased stomatal conductance (Elcan and Pezeshki, 2002; Smith and Huslig, 1990) may result in greater sensitivity of plants to water deficit after waters recede.

Some plants use compensatory mechanisms to tolerate flooding by optimizing ethanol fermentation when exposed to anoxic conditions. At the onset of flooding, alcohol dehydrogenase is stimulated and results in the production of ethanol (Chung and Ferl, 1999). Even though many wetland plants use ethanol fermentation under anoxic conditions, some plants will use an alternative pathway to produce alanine, lactate, or malate (Ernst, 1990). Anaerobic respiration uses a large sum of energy and in order to maintain cellular function, a large storage of carbohydrates needs to be readily available. In the situation where leaves are not capable of providing a sufficient oxygen supply to the roots, plants will store carbohydrates in their rhizomes for future use.

In addition to surviving in low-oxygen conditions, plants must have adaptions to resist stress from water deficit in areas where flooding is ephemeral (Braendle and Crawford, 1999). Plants exhibit stress from water deficit by a reduction in growth, wilting of leaves, discoloration of leaves, and reduced speed of recovery of stem water potential after irrigation (Dickson et al., 1965; Dickson and Broyer

1972; Rosenthal et al., 1987). Leaf development (leaf number, extension, and senescence) can also be affected by water deficit (Rosenthal et al., 1987). Dickson and Broyer (1972) found seedlings of *N. aquatica* avoid conditions of water deficit by reducing transpiration rates through closure of stomata, leaf abscission, and increasing root : shoot. Wetland plants can tolerate water deficit by increasing cellular elasticity and osmotic regulation (Nash and Graves, 1993; Romanello et al., 2008; Touchette et al., 2007). Although physiological mechanisms allow for survival of plants at low water potentials, some may not be desirable aesthetic attributes (excessive leaf abscission) for plants used in managed landscapes (Zollinger et al., 2006).

Tolerance of *N. biflora* and *N. sylvatica* to water stress

Many authors have concluded that *N. sylvatica* var. *biflora* is extremely tolerant of flooding and even thrives under those conditions (Applequist, 1960; Hook et al., 1970a; Hook et al., 1970b; Hook et al. 1971; Hook, 1984). Hook et al. (1970b) conducted an experiment to test the effects of water regimes on growth of seedlings of *N. sylvatica* var. *biflora*. Hypertrophied lenticels were formed on submerged stems after two weeks of flooding. Root : shoot decreased with the increased degree of flooding and growth of plants was positively correlated with pO_2 and negatively with pCO_2 in the water of the soil. Discoloration of leaves (similar to fall color), leaf abscission, and leaf wilting was prevalent in all treatments of flooding. Height growth doubled and total dry weight of plants was two to five times greater in moving water than when grown in stagnant water. They found that stagnant water results in death of the initial root system and reduces the development of adventitious roots. In accordance with results from Hook's experiment, Harms (1973) concluded that growth and dry weight of *N. sylvatica* var. *biflora* were poorest in deep-stagnant water compared with surface-flooded moving water.

Hook et al. (1971) conducted an experiment to study the mechanisms of flood tolerance of *N. sylvatica* var. *biflora*. They found that under conditions of flooding, the newly initiated roots of *N. sylvatica* var. *biflora* increased production of ethanol and lactic acid; these roots were also capable of

oxidizing rhizosphere. Flooded roots produced more ethanol than unflooded roots; unflooded roots did not oxidize their rhizosphere. The authors suspected that oxygen entered the stems through the lenticels and was transported through the cortex or phloem. Roots of *N. sylvatica* var. *biflora* tolerated 10% CO_2 without deleterious effects, but 31% CO_2 around the roots resulted in oxidation of the rhizosphere by the new roots. They concluded that the collective adaptions of accelerated anaerobic respiration in the absence of oxygen, oxidation of the rhizosphere, and tolerance to CO_2 of new roots explain the tolerance to flooding of *N. sylvatica* var. *biflora*.

Keeley and Franz (1979) conducted experiments to study the adaptive strategies of *N. sylvatica* var. *sylvatica* and *N. sylvatica* var. *biflora* to flooding. They concluded the adaption to flooding of *N. sylvatica* var. *biflora* is a sequential process with changes in plant metabolism and anatomy. Following one week of flooding, the root systems of seedlings of *N. sylvatica* var. *biflora* started to deteriorate, but were replaced by roots that were morphologically distinct. The roots of this new root system were brittle, and several millimeters in diameter. After one month of flooding, seedlings of *N. sylvatica* var. *biflora* were capable of producing large amount of ethanol as a result of the newly-formed root system. Between one month and one year of flooding, another change occurred in the root system. The succulent roots formed during the first change were replaced with another root system that resembled roots of plants in well-drained treatments. The new root system showed an increase in transportation of oxygen compared with plants in drained and flooded one month treatments. The authors suspected the new roots were probably morphologically distinct from the second ones by having larger air spaces, which increased the internal transport of gas in the plant. Thus, the short-term response to flooding is a compensatory strategy by producing roots that are capable of enhanced rates of anaerobic respiration; plants subjected to prolonged hypoxia switch to a root architecture that supports aerobic respiration. In the same study, the ethanol producing capacity of *N. sylvatica* var. *sylvatica* increased compared to that of plants in the well-drained treatment immediately after flooding. After one month, ethanol

production began to decrease and a much steeper drop in ethanol production occurred between one month and one year of flooding. They noted that the secondary root systems of the plants in the flooded treatments began to deteriorate after one month and were highly necrotic after one year. They concluded that the drop off in ethanol producing capacity was due to the deterioration of the root system and that *N. sylvatica* var. *sylvatica* is not tolerant of flooding. Nash and Graves (1993) also concluded *N. sylvatica* var. *sylvatica* is sensitive to flooding and extreme drought. They conducted an experiment to study the effects of flooding on *N. sylvatica* among other species of woody plants. Treatments of flooding reduced net assimilation rate (NAR) and relative growth rate (RGR) of *N. sylvatica*. After 118 days of flooding, 33% of plants of *N. sylvatica* were dead. Root: shoot of *N. sylvatica* was lower for plants in the flooded treatment compared to plants maintained at container capacity.

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CHAPTER 2. PRETREATMENT EFFECTS ON SEED GERMINATION OF TWO *NYSSA* SPECIES

A manuscript intended for submission to *HortScience*

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Abstract. The genus *Nyssa* L. includes several woody species with ornamental traits valued by horticulturists, but only black gum (*Nyssa sylvatica* Marsh.) is prevalent in the nursery trade. A congener, swamp tupelo (*Nyssa biflora* Walt.) could become a marketable shade tree, but little is known about propagating it from seeds. Because cold, moist stratification overcomes embryo dormancies of black gum, we compared germination of cleaned seeds (depulped seeds with endocarp intact) of swamp tupelo and black gum that were moist-stratified at 5 °C for 0, 14, 28, 42, 56, 70, 84, and 112 days. Seeds of swamp tupelo within intact drupes also were subjected to these treatments. Across all times of stratification, 11.1% and 78.6% of seeds of swamp tupelo germinated with pulp intact and with pulp removed, respectively. Germination value of depulped seeds of swamp tupelo increased from 1.26 to 3.23 as time of stratification increased. Although depulped seeds of black gum responded similarly, the benefit of stratification was more pronounced, and the mean germination percentage was lower than for swamp tupelo (66% vs. 79%). We conclude that seeds of swamp tupelo are less resistant to germination than are seeds of black gum. In a second experiment, germination of seeds of basil

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(*Ocimum basilicum* L. 'Superbo'), spinach (*Spinacea oleracea* L. 'Bloomsdale'), zinnia (*Zinnia* ×*marylandica* Spooner, Stimart, & Boyle 'Double Zahara Cherry'), and swamp tupelo was quantified after treatments of irrigation with two concentrations of a slurry of fruit pulp of swamp tupelo. Germination value of seeds across all species irrigated with deionized water was 206.5 times that of seeds irrigated with the more concentrated slurry. Results from both experiments suggest the pulp that surrounds the seeds of swamp tupelo contains germination inhibitors; propagators therefore should depulp the seeds before sowing.

Introduction

Swamp tupelo (*Nyssa biflora*) is indigenous to swamps and bottomlands of the southeastern United States. The species distribution comprises the Coastal Plain from Delaware to south Florida and east Texas, and extends north to southern Illinois. In contrast, black gum (*Nyssa sylvatica*) is indigenous to much of the eastern United States and occurs from Maine to southern Ontario, to central Missouri, and to east Texas and southern Florida. The two species rarely occur together but can be challenging to differentiate (Outcalt, 1990). Although swamp tupelo is not marketed for landscape use, black gum is promoted in the industry as a specimen tree. Individuals of black gum can form strong central leaders that give rise to distinctively stratified scaffold limbs that bear glossy, colorful foliage. Black gum is considered one of the most beautiful trees native to North America, and cultivars have been selected for outstanding autumnal foliar color and desirable canopy architecture (Dirr, 2009).

Taxonomic confusion may help to explain why swamp tupelo has not become a nursery crop. Swamp tupelo has been regarded by some as a variant of black gum [*Nyssa sylvatica* var. *biflora* (Walt.) Sarg.], although embryological studies suggest swamp tupelo is more closely related to water tupelo (*Nyssa aquatica* L.) than to black gum (Tandon and Herr, 1971). A recent taxonomic revision concluded

that swamp tupelo is one of five species of *Nyssa* indigenous to North America and is distinct from black gum (Burckhalter, 1992).

Naturally occurring in soils that are waterlogged for the majority of the growing season (Penfound, 1952; Applequist, 1956), swamp tupelo may be useful when planted in landscapes where fluctuations in soil moisture and aeration are common. Unlike swamp tupelo, wild plants of black gum are not restricted to wetlands but instead occur on a soil-moisture gradient of dry uplands to alluvial stream bottoms and occasionally on edges of swamps (McGee, 1990). Plants that occupy sites in nature that are flooded seasonally or continuously not only resist stresses that afflict other species in inundated soils, but also may tolerate or resist stress from water deficit (Li and Zhong, 2006). The popular tree bald cypress (*Taxodium distichum* L.) is one of numerous species important for horticulture that illustrate the tendency for flood-resistant trees to be resistant to drought as well. Within the genus *Nyssa*, differences in their occurrence in nature underscore the possibility that the highly flood-tolerant swamp tupelo may respond more favorably than black gum to a wide range of soil moisture contents. Aesthetic features of swamp tupelo include glossy green summer foliage and scarlet-red autumnal color.

Dirr and Heuser (2006) noted that the removal of pulp from seeds of black gum does not appear to be necessary for germination to occur. Although effects of intact pulp on germination of seeds of *Nyssa* is unknown, studies on other species suggest compounds in fruit pulp may inhibit germination. Fagan et al. (1981) demonstrated that seeds of lily turf [*Liriope muscari* (Decne.) L.H. Bailey] with the mealy covering intact had a lower germination rate than seeds with coverings removed. Norton (1980) found similar effects of removing the aril of seeds of southern magnolia (*Magnolia grandiflora* L.). Kobayashi et al. (2010) suggested accumulation of abscisic acid (ABA) in the flesh of fruit of watermelon [*Citrullus lanatus* (Thunb.) Mansf.] may prevent viviparous germination. Cipollini and Levey (1997) conducted experiments with fruits of spicebush (*Lindera benzoin* L.) to test effects of pulp on

germination. Seed germination was approximately 100% when fruits were hand-cleaned or regurgitated by American robins (*Turdus migratorious* L.), but was below 1% when seeds remained within intact fruits. Thus, the pulp of other drupaceous fruits may contain compounds that inhibit germination.

Protocols for propagation of swamp tupelo from seed have not been reported, which may be contributing to the absence of the species in horticultural commerce. Although requirements of stratification can vary with seed provenance, excellent germination of seeds of black gum can generally be achieved with treatments of stratification for three months at 40° F (Dirr and Heuser, 2006). Based on its distribution in regions with relatively mild winters, our hypothesis was that seeds of swamp tupelo are less resistant to germination, thus requiring shorter durations of stratification to release embryo dormancy, than seeds of black gum.

We conducted an experiment to determine 1) if removal of pulp from seeds of swamp tupelo improves germination responses, and 2) the time of stratification necessary for increased speed, synchrony, and total germination of seeds of swamp tupelo. Treatments were designed based on protocols that promote germination of black gum, and seeds of both swamp tupelo and black gum were studied. We compared germination of cleaned seeds (depulped seeds with endocarp intact) and seeds within intact drupes of swamp tupelo over a range of stratification periods to test for effects of pulp removal. In addition, we compared swamp tupelo with black gum to determine how time of stratification affects germination of cleaned seeds. In a second experiment, we compared germination of seeds of basil (*Ocimum basilicum* 'Superbo'), spinach (*Spinacea oleracea* 'Bloomsdale'), zinnia (*Zinnia* ×*marylandica* 'Double Zahara Cherry'), and swamp tupelo within intact drupes and cleaned seeds of swamp tupelo after irrigation with different concentrations of slurry of fruit pulp of swamp tupelo. The objective of this experiment was to determine if germination of seeds of species in various plant families is inhibited by the fruit pulp of swamp tupelo.

Materials and Methods

Experiment 1. During Oct. and Nov. of 2013, drupes of swamp tupelo were collected from trees indigenous to two sites in VA, the margin of Lake Prince in Suffolk, and Seacock Chapel Swamp in Zuni. Additional drupes of swamp tupelo were collected from trees indigenous to Hoke County, NC. Drupes of black gum were collected from a cultivated tree at the Minnesota Landscape Arboretum, Chanhassen, MN. Fruits were stored in polyethylene bags in a dark cooler at 5 °C before cleaning and removal of the pulp. Natural softening of the fruit can be achieved by storage in polyethylene bags, resulting in easy removal of the pulp (Dirr and Heuser, 2006). Seeds were depulped by maceration after submersion of fruits in water. Cleaned seeds were stored in polyethylene bags in a dark cooler at 5 °C until initiation of treatments.

Cold, moist stratification of multiseed experimental units of 20 cleaned seeds or seeds within intact fruit was initiated on 22 Nov., 2013. Each group of 20 was placed in a polyethylene bag (11 x 15 cm) that contained 100 cm^3 of coarse sand that was moistened in advance by blending 400 ml of tap water per 454g of dry sand. Bags with seeds were held in a dark cooler at 5 °C except for those randomly assigned to receive no stratification. Five bags were assigned randomly to each species-stratification combination. Of the five bags with seeds of swamp tupelo, three contained seeds from Lake Prince, one contained seeds from Seacock Chapel Swamp, and one contained seeds from Hoke County.

Bags were removed from the cooler at treatment durations of 14, 28, 42, 56, 70, 84, and 112 d. The control was placed in the germination chamber on Nov. 22 and received no stratification. Cleaned seeds or seeds within intact fruits were removed from the stratification medium and rinsed with tap water to remove residual particles of sand. Twenty seeds or seeds within intact fruits from the same bag were placed on two sheets of 12-ply Versa-Pak™ Cellulose Wadding (K-24; Blue Ridge Tissue Corp.,

Lenoir, NC, U.S.) in a transparent, plastic-hinged germination box that was 16 cm x 27 cm x 4 cm and wetted with 200 g of tap water. Boxes were randomly assigned a position on 12 shelves in a growth chamber with five boxes on each shelf. The growth chamber was equipped with six cool-white fluorescent lamps and programmed at alternating day/night temperatures of 30/20 °C for 8/16 h, respectively.

Treatment effects on germination, defined as the initial emergence of the radicle during 56 d in the growth chamber, were examined by calculating several response variables. The number of germinated seeds was determined daily. Mean daily germination was determined as the germination percentage per experimental unit (germination box) divided by 56, the number of days of the germination period. Peak value was the cumulative germination percentage for each experimental unit on the day when the largest number of seeds germinated (peak day), divided by the number of days required to reach that germination percentage. Germination value was calculated as the product of peak value and mean daily germination and is a composite expression of the rate and completeness of germination (Czabator, 1962). Germination percentage was determined on day 56. Experimental units in which no seeds germinated were ascribed a 56, the poorest score possible for germination distribution and peak day. Germination distribution was the number of days between the first and last days germination occurred in experimental unit. Upon germination, individual seedlings were removed from boxes.

Tetrazolium (2, 3, 5-triphenyltetrazolium chloride) tests of viability (Peters, 2000) were completed on ungerminated seeds at the end of the germination period. Protocols from the ASOSA/SCST Tetrazolium Testing Handbook (2010) were followed with slight modifications. Distal ends of the cotyledons of ungerminated seeds were removed, and seeds were soaked in 1.0% solution of tetrazolium for 12 hr in an oven at 35° C. Following removal from the oven, embryos and endosperms were visually deemed viable, not viable, or absent.

Effects of species, time of stratification, and presence of pulp were determined by analysis of variance (ANOVA) and Tukey's honestly significant difference option of JMP® 11 software (JMP®, Version 11. SAS Institute Inc., Cary, NC, U.S., 1989-2007). Data from cleaned seeds of swamp tupelo and black gum were analyzed together to assess the effects of species, stratification, and the interaction of species and stratification. An analysis was completed separately on data from cleaned seeds and intact drupes of swamp tupelo to assess the effects of stratification, pulp, and the interaction of stratification and pulp.

Experiment 2. A slurry of 1300 ml of pulp of swamp tupelo fruits was generated by macerating fruits in deionized water at the ratio of 20 ml of water to 50 fruits and strained to remove exocarps and seeds. The initial slurry was diluted by adding two parts of deionized water to one part of the initial slurry and deemed the most concentrated (high) treatment. The weaker treatment (low) was created by adding four parts of deionized water to one part of the initial slurry; deionized water was used as the control. Ten seeds of either basil, spinach, zinnia, swamp tupelo within intact fruits, or cleaned seeds of swamp tupelo, were sowed in containers with heights of 7.5 cm, top diameters of 8.5 cm, and bottom diameters of 6.5 cm, filled with LC1 peat-based growing medium (Sunshine® Mix #1; Sun Gro Horticulture, Agawan, MA, U.S.). Containers were randomly arranged on one shelf of a growth chamber and conditions of the chamber were the same as in Experiment 1. On day 0 of the experiment, containers were irrigated with 80 ml of one of the three treatments. Subsequent irrigations of 15 ml occurred on days 5 and 6. Following the final irrigation on day 6, containers were placed in plastic ziplock bags and sealed to prevent further desiccation of the medium. Germination was defined as the visual emergence of the hypocotyl and was recorded daily. The same response variables used in Experiment 1 were calculated in Experiment 2. Effects of species, treatment, and the interaction of species and treatment was ANOVA and Tukey's honestly significant difference option of JMP® 11 software (JMP®, Version 11. SAS Institute Inc., Cary, NC, U.S., 1989-2007).

Results

Experiment 1. Main effects for cleaned seeds of swamp tupelo and black gum. Analysis of variance detected effects of species on germination value ($P \leq 0.0031$), germination percentage ($P \leq 0.0001$), peak day ($P \leq 0.0001$), peak value ($P \leq 0.0001$), and mean daily germination ($P \leq 0.0001$), but not germination distribution. Stratification affected germination value ($P \leq 0.0001$), germination percentage ($P \leq 0.0001$), germination distribution ($P \leq 0.031$), peak day ($P \leq 0.0001$), peak value ($P \leq 0.0001$), and mean daily germination ($P \leq 0.0001$). An interaction of species and stratification for germination value ($P \leq 0.0199$), germination percentage ($P \leq 0.0001$), peak day ($P \leq 0.0001$), peak value ($P \leq 0.0103$), and mean daily germination ($P \leq 0.0001$) was detected, but not for germination distribution.

Experiment 1. Main effects for cleaned seeds and seeds within intact fruits of swamp tupelo. Pulp influenced germination value ($P \leq 0.0001$), germination percentage ($P \leq 0.0001$), germination distribution ($P \leq 0.0258$), peak day ($P \leq 0.0001$), peak value ($P \leq 0.0001$), and mean daily germination ($P \leq 0.0001$). Stratification influenced germination value ($P \leq 0.0005$) and peak value ($P \leq 0.0003$). An interaction of stratification and pulp for germination value ($P \leq 0.0053$) and peak value ($P \leq 0.0004$) was detected, but not for germination percentage, germination distribution, peak day, or mean daily germination.

Experiment 1. Species effects. Averaged over all times of stratification, germination percentage of cleaned seeds of swamp tupelo was 1.2 times that of cleaned seeds of black gum (Table 1). The peak day of germination of cleaned seeds of swamp tupelo occurred 6.6 d earlier than cleaned seeds of black gum (Table 1). Peak value of seeds of swamp tupelo was 1.4 times that of cleaned seeds of black gum (Table 1). Mean daily germination of cleaned seeds of swamp tupelo was 1.2 times that of cleaned

seeds of black gum (Table 1). Germination value and distribution were not different for cleaned seeds of swamp tupelo and black gum (Table 1).

Experiment 1. Pulp effects. Averaged over all times of stratification, germination value of cleaned seeds of swamp tupelo was 217 times that of seeds within intact fruits of swamp tupelo (Table 1). Germination percentage of cleaned seeds of swamp tupelo was 7.1 times that of seeds within intact fruits of swamp tupelo (Table 1). Germination distribution of cleaned seeds of swamp tupelo was 8.4 d less than that of seeds within intact fruits of swamp tupelo (Table 1). The peak day of germination of cleaned seeds of swamp tupelo occurred 32.3 d earlier than did seeds within intact fruits of swamp tupelo (Table 1). Peak value of cleaned seeds of swamp tupelo was 37 times that of seeds within intact fruits of swamp tupelo (Table 1). Mean daily germination of cleaned seeds of swamp tupelo was seven times that of seeds within intact fruits of swamp tupelo (Table 1).

Experiment 1. Effects of time of stratification. Time of stratification did not affect germination value, germination percentage, germination distribution, peak day, peak value, or mean daily germination of seeds of swamp tupelo within intact fruits (Table 2). Germination percentage, germination distribution, peak day, and mean daily germination of cleaned seeds of swamp tupelo were also unaffected by increasing time of stratification, however, germination value and peak value were affected (Table 2). Linear regression functions best represent the relationship of time of stratification and germination value (Fig. 1) and peak value. Germination value of cleaned seeds of swamp tupelo was as low as 1.26 at 0 d and as high as 3.23 at 112 d of stratification (Table 2). Peak value of cleaned seeds of swamp tupelo ranged from 4.89 at 0 d to 10.71 at 112 d of stratification (Table 2).

Germination value, germination percentage, germination distribution, peak day, peak value, and mean daily germination of cleaned seeds of black gum were all affected by time of stratification. Germination value (Fig. 1), germination percentage, peak value, and mean daily germination increased

linearly with increasing time of stratification (Table 2). Germination value was <0.01 at 0 d of stratification and increased to 3.49 with 112 d of stratification (Table 2). Germination percentage was as low as 1 with 0 d of stratification and was as high as 96 with 112 d of stratification (Table 2). Germination distribution was the largest at 0 d of stratification and decreased to 17.4 with 112 d of stratification (Table 2). The peak day was as late as day 50.6 with 0 d of stratification and decreased to day 7.2 with 112 d of stratification (Table 2). Mean daily germination was 0.01 with 0 d stratification and increased to 1.71 with 112 d of stratification (Table 2).

Experiment 1. Tetrazolium tests. Tetrazolium tests revealed ungerminated seeds of swamp tupelo within intact fruits over all periods of stratification were 81.4% viable and 5.9% not viable; embryos were absent in 12.7% of seeds. Ungerminated cleaned seeds of swamp tupelo were 41.5% viable, 25.2% not viable, and 33.3% lacked an embryo. Ungerminated cleaned seeds of black gum were 90.5% viable, 2.6% not viable, and 6.9% lacked an embryo.

Experiment 2. Main effects. Species affected germination value ($P \leq 0.0137$), germination percentage ($P \leq 0.0004$), peak day ($P \leq 0.039$), peak value ($P \leq 0.0026$), and mean daily germination ($P \leq 0.0005$), but not germination distribution. Treatment affected germination value ($P \leq 0.0003$), germination percentage ($P \leq 0.0001$), germination distribution ($P \leq 0.0001$), peak day ($P \leq 0.0001$), peak value ($P < 0.0001$), and mean daily germination ($P \leq 0.0001$). An interaction of species and treatment was detected for germination value ($P \leq 0.0001$), germination percentage ($P \leq 0.0001$), germination distribution ($P \leq 0.0003$), peak day ($P \leq 0.0001$), peak value ($P \leq 0.0001$), and mean daily germination ($P \leq 0.0001$).

Experiment 2. Treatment effect. Germination value of the control was 12 times that of that of the low treatment and 413 times that of the high treatment (Table 3). Germination percentage of the control was 3.3 times that of the low treatment and 18.2 times that of the high treatment over all

species (Table 3). Germination distribution of the control was 11 d less than the low treatment and approximately 26.5 d earlier than the high treatment (Table 3). The peak day of the control occurred nearly 13.5 d earlier than that of the low treatment and 28.9 d earlier than that of the high treatment (Table 3). Mean daily germination of the control treatment was 3.3 times that of the low treatment and 16.5 times that of the high treatment (Table 3).

Discussion

Our results are significant to horticulture in three ways. First, we have shown that cold stratification enhances the peak value of cleaned seeds of swamp tupelo on peak day but does not affect the germination percentage or total germination. Second, our hypothesis that seeds of swamp tupelo are less resistant to germination than seeds of black gum was supported. Lastly, we have shown that the fruit pulp of swamp tupelo inhibits germination of seeds of swamp tupelo and other species. We conclude that swamp tupelo can be propagated successfully from seed which would allow for selection from a wide array of genotypes for desired ornamental or physiological traits. We recommend that staminate trees of this polygamodioecious species should be selected to avoid problems associated with messy fruits.

The germination value which is the best single measure of quality and germinability of seeds of woody plants (Czabator, 1962, Hartmann et. al, 1990), and peak value, representing the vigorousness of germination on the peak day, was highest with 112 days of stratification (Table 2). If total germination is the objective of the propagator, we do not recommend stratifying seeds of swamp tupelo, but vigorousness of germination can be enhanced by stratification of seeds for 112 days. Linear regression functions best represent the relationship between time of stratification and the germination value of cleaned seeds of swamp tupelo and black gum as shown in Fig. 1. Additionally, the interaction of species and stratification can be observed by the difference in trend lines between species as shown in Fig. 1. Although the peak value is increased by stratification, the total germination, germination

distribution, peak day, and mean daily germination were unaffected by increasing time of stratification (Table 2). Since less than half of the ungerminated cleaned seeds of swamp tupelo were viable, there may have been additional reasons for a lack of germination besides an unviable embryo. Stratification had a more pronounced effect on cleaned seeds of swamp tupelo compared to cleaned seeds of black gum (Table 2). Time of stratification increased germination value, germination percentage, peak value, and mean daily germination, but decreased the germination distribution and peak day of cleaned seeds of black gum. Using the germination value as the composite expression of germination, we concluded that germination of cleaned seeds of black gum was optimized when stratified for 112 days (Table 2). Since 90.5% of cleaned seeds of black gum were viable, it is possible that seeds treated with longer times of stratification would have resulted in germination of the viable seeds. Although the peak value of cleaned seeds of black gum was affected by time of stratification, the effect on peak day was less pronounced (Table 2). This is due to an increase in germination percentage on the peak day with increasing time of stratification. The range of the swamp tupelo is restricted to the southeastern United States where winters are relatively shorter and less harsh which may explain why seeds of swamp tupelo require a shorter period of chilling than those of black gum.

Stratification had no effect on the germination responses of seeds of swamp tupelo within intact fruits. Germination of seeds within intact fruits of swamp tupelo was likely restricted by the presence of the pulp since 81.4 % of those seeds were viable. Evenari (1949) and Mayer and Poljakoff-Mayber (1989) noted that fruits can contain germination inhibitors. When pulp was not removed from seeds of swamp tupelo, germination was inhibited to an extent that would be undesirable to propagators. The restricted germination may be due to chemical inhibitors or unsuitable water relations caused by the intact pulp. Kobayashi et al. (2010) concluded the presence of ABA in fruit pulp of watermelon may contribute to restricted germination. ABA plays a dynamic role in events during seed development such as prevention of precocious germination and induction of primary dormancy; thus, ABA in the fruit pulp

of swamp tupelo may inhibit germination. Although seeds were pooled from multiple trees within a population in our study, differences in germination responses of seeds within intact fruits can be detected in other species of woody plants (Meyer and Witmer, 1998). Differences in nutrient composition of fruits (Cipollini and Stiles, 1992), and the amount of fruit pulp covering the seed may help to explain variation in germination due to presence of fruit pulp (Barnea et al., 1991; Howe, 1980; Howe and Vande Kerckhove, 1981; Izhaki and Safriel, 1990). Fruit pulp and exocarps with relatively thick cuticular layers can inhibit germination by their high osmotic pressure from dissolved sugars and reduced permeability of water which are factors that affect water relations of seeds, thus, affecting germination (Mayer and Poljakoff-Mayber, 1989). Schrader and Graves (2000) also detected variation in germination responses between trees within populations of seaside alder [*Alnus maritima* (Marsh) Nutt.]. We did not test for effects of seed provenance on germination; geographic and ecotypic differences were not considered. Because seeds of black gum were collected from a cultivated plant, our study does not represent the range of genetic diversity of the species. Additionally, seeds of swamp tupelo were not collected from trees across the distribution of the species. Stratification effects may or may not differ between seeds collected from trees at northern latitudes of the range compared to seeds collected at southern latitudes (Bischoff et al., 2006). Future research should involve testing for effects of provenance on seeds of swamp tupelo. In our second experiment, we found that germination of seeds of others species is inhibited by the fruit pulp of swamp tupelo, supporting results from Expt. 1 (Table 3). Although our objective was not to determine which species was more affected by inhibitors in fruit pulp of swamp tupelo, we discovered that basil and zinnia are more sensitive than spinach to the inhibitors of fruit pulp of swamp tupelo as shown in Fig. 2.

Results from our study support our hypothesis that pulp of fruits of swamp tupelo contain germination inhibitors but we did not investigate the inhibition effects of fruit pulp of black gum. Future studies can be conducted to discover if the same trend occurs with fruit pulp of black gum. Although

more studies should be completed to assess whether swamp tupelo may warrant use as a plant in managed landscapes, our results provide propagators interested in growing swamp tupelo with beneficial information on propagation from seeds. Because naturally occurring populations of swamp tupelo are restricted to the southeastern United States, it is important to investigate the cold hardiness limits of the species before it is marketed as a horticultural plant.

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Table 1. Comparison of germination responses of cleaned seeds of swamp tupelo (*N. biflora*) and black gum (*N. sylvatica*) and cleaned seeds and seeds within intact fruits of swamp tupelo across all times of stratification. Cleaned seeds and seeds within intact fruits were stratified for 0, 14, 28, 42, 56, 70, 84, and 112 d in the dark at 5°C. Values for species/pulp combinations are means of 40 multiseed replications.

		Germination			Peak		Mean daily germination
Main effects	Cleaned	Value	%	Distribution (d)	Day	Value	(%/d)
Species							
swamp tupelo	Y	2.17 A[z]	78.6 A	16.9 A	6.9 B	7.4 A	1.4 A
black gum	Y	1.68 A	65.8 B	20.9 A	13.5 A	5.4 B	1.2 B
Pulp							
swamp tupelo	N	0.01 b[y]	11.1 b	25.3 a	39.2 a	0.2 b	0.2 b
swamp tupelo	Y	2.17 a	78.6 a	16.9 b	6.9 b	7.4 a	1.4 a

[z]Means of species/pulp combinations within the same column followed by the same capital letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

[y]Means of species/pulp combinations within the same column followed by the same lower-case letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

Table 2. Comparison of germination responses of species/treatment combinations at each level of time of stratification.

Values are means of five multiseed replications.

Time of stratification of species/treatment combinations	Germination			Peak		Mean daily germination
	Value	%	Distribution (d)	Day	Value	(%/d)
swamp tupelo (not cleaned)						
0	0.01 A[z]	11 A	31.6 A	48.4 A	0.15 A	0.20 A
14	0.01 A	8 A	6.2 A	31.6 A	0.18 A	0.14 A
28	<0.01 A	5 A	24.4 A	42.0 A	0.12 A	0.10 A
42	<0.01 A	6 A	12.4 A	53.0 A	0.10 A	0.11 A
56	0.02 A	18 A	31.2 A	34.2 A	0.21 A	0.32 A
70	0.02 A	14 A	23.4 A	35.8 A	0.30 A	0.25 A
84	0.01 A	11 A	40.6 A	37.6 A	0.14 A	0.20 A

Table 2 continued

112	0.02 A	16 A	32.8 A	31.0 A	0.21 A	0.29 A
swamp tupelo						
(cleaned)						
0	1.26 B	71 A	18.8 A	8.4 A	4.89 C	1.27 A
14	1.41 B	79 A	23.8 A	5.0 A	5.00 BC	1.41 A
28	1.73 AB	69 A	12.6 A	10.2 A	6.00 B	1.23 A
42	3.02 AB	90 A	16.2 A	5.8 A	9.39 AB	1.61 A
56	2.07 AB	79 A	19.8 A	6.2 A	7.33 ABC	1.41 A
70	2.41 AB	78 A	18.4 A	5.8 A	8.33 ABC	1.39 A
84	2.26 AB	80 A	19.6 A	6.6 A	7.81 ABC	1.42 A
112	3.23 A	83 A	5.6 A	7.0 A	10.71 A	1.48 A
black gum (cleaned)						
0	<0.01 D	1 C	45.0 A	50.6 A	0.03 D	0.01 C
14	0.04 D	16 C	14.2 B	12.2 B	0.76 D	0.29 C
28	1.01 CD	67 B	11.8 B	9.8 B	4.15 C	1.20 B
42	1.81 BC	85 A	28.2 AB	7.0 B	5.94 BC	1.52 A
56	2.20 B	86 A	20.2 AB	7.4 B	7.15 B	1.54 A

Table 2 continued

70	2.81 AB	94 A	15.8 B	6.0 B	8.33 AB	1.68 A
84	2.10 B	81 AB	14.6 B	7.4 B	7.03 B	1.45 AB
112	3.49 A	96 A	17.4 AB	7.2 B	10.13 A	1.71 A

[z]Means of species/treatment combinations within a column followed by the same capital letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

Table 3. Comparison of germination responses of seeds irrigated with two concentrations of slurry of fruit pulp of swamp tupelo (*N. biflora*) and deionized water (control) across all species. There were 20 multiseed replications per treatment.

	Germination			Peak		Mean daily germination
Treatment	Value	%	Distribution (d)	Day	Value	(%/d)
Control	41.3 A[z]	45.5 A	15.8 B	16.3 B	7.7 A	3.3 A
Low	3.4 B	14.0 B	26.8 AB	29.8 AB	1.3 B	1.0 B
High	0.1 B	2.5 B	42.3 A	45.2 A	0.2 B	0.2 B

[z]Treatment means across all species within each column followed by the same letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

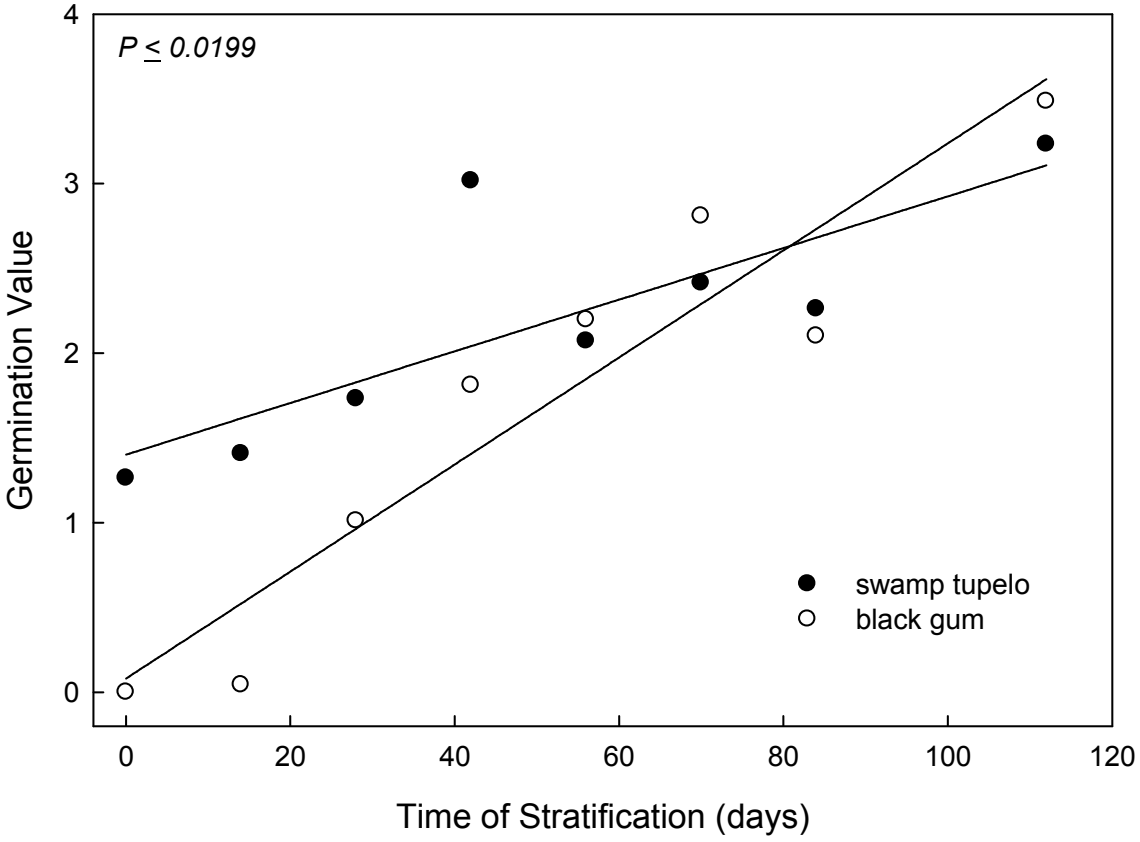

Fig. 1. Germination value of cleaned seeds of swamp tupelo (*N. biflora*) and black gum (*N. sylvatica)* represented by linear regression. Germination value (swamp tupelo: y = 0.0152x + 1.4019, r^2 = 0.65, $P \leq$ 0.0164; black gum: y = 0.0316x + 0.0809, r^2 = 0.89, $P \leq 0.0004$). Values are means of five multiseed replications. A $P \leq 0.05$ in the upper left hand corner of the figure indicates that two lines better represent the means than one according to a two degrees of freedom test.

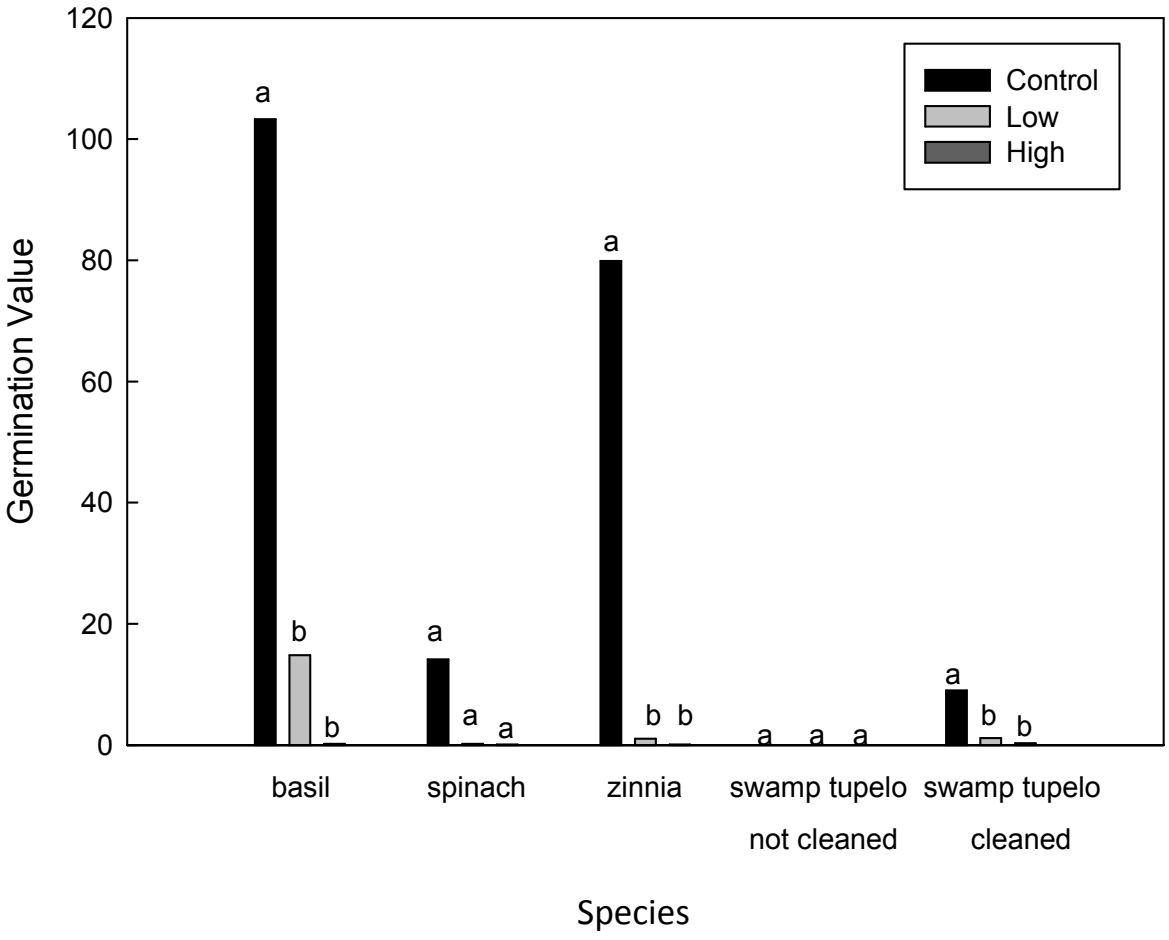

Fig. 2. Germination responses of seeds of basil (*Ocimum basilicum* 'Superbo'), spinach (*Spinacea oleracea* 'Bloomsdale'), zinnia (*Zinnia* ×*marylandica* 'Double Zahara Cherry'), and cleaned seeds or seeds within intact fruits of swamp tupelo (*N. biflora*) at each level of irrigation treatment. Treatments within a species represented by the same letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

CHAPTER 3. *NYSSA BIFLORA* IS MORE RESISTANT TO WATER STRESS THAN *NYSSA SYLVATICA*

A manuscript intended for submission to *HortScience*

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Additional index words. Drought, flooding, aerenchyma, nursery crops, Cornaceae

Abstract. Several woody taxa indigenous to wetlands resist stress from both drought and flooding. Swamp tupelo (*Nyssa biflora* Walt.) is native to swamps and bottomlands of the southeastern United States. Although its congener, black gum (*Nyssa sylvatica* Marsh.) is the only member of the genus that is widely produced in nurseries, we hypothesized that swamp tupelo may be better suited for managed landscapes where extreme soil-moisture contents are common stressors. We compared responses of containerized seedlings of swamp tupelo and black gum treated with six root-zone moisture regimes in a greenhouse. Treatments ranged from complete inundation to severe drought (3% root-zone water by volume). All plants of swamp tupelo survived, whereas four of six plants of black gum died when treated with complete inundation. Rate of dry mass accumulation and increase in height of swamp tupelo were two times and 1.4 times those of black gum, respectively, across all treatments. Rates of photosynthesis of swamp tupelo subjected to drought for two weeks were higher than those of black gum ($P \leq 0.002$) and were maximal with 0.30 $m^3\ m^{-3}$ root-zone moisture. The tolerance to flooding of swamp tupelo coincided with formation of adventitious roots and hypertrophied lenticels. We conclude that seedlings of swamp tupelo are more resistant to water stress than seedlings of black gum;

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additional studies with swamp tupelo are warranted to assess its merit as nursery crop marketed for use in managed landscapes.

Introduction

Black gum (*Nyssa sylvatica*) is a nursery crop marketed as a specimen tree grown for its distinct growth habit and glossy, colorful foliage. Many cultivars of black gum have been selected for autumnal color of foliage and desirable canopy architecture (Dirr, 2009). Black gum is indigenous to uplands and in alluvial stream bottoms throughout most of the eastern U.S. (McGee, 1990). A congener of black gum, swamp tupelo (*Nyssa biflora*), is indigenous to swamps and bottomlands of the Coastal Plain but has not been introduced to horticultural trade. Some authors (Eyde, 1963) consider swamp tupelo a variant of black gum that can hybridize with black gum, but a taxonomic revision classified swamp tupelo as a distinct species (Burckhalter, 1992). Taxonomic confusion may help to explain why swamp tupelo has not been investigated for potential as a horticultural plant.

Unlike black gum, swamp tupelo grows in soils that are waterlogged most of the growing season and is considered extremely tolerant of flooding (Hook et al., 1970b; Hook et al., 1971). Harms (1973) found that growth of swamp tupelo is greatest when growing in shallow, moving water and that growth can be reduced by 50% in stagnant water. Morphological changes to roots and stems are the suspected adaptive mechanisms that explain survival of swamp tupelo in waterlogged soils (Hook et al., 1970a; Hook et al., 1971). Seedlings of swamp tupelo develop hypertrophied lenticels and adventitious roots on submerged stems (Hook and Stubbs, 1967; Hook et al., 1970a**;** Hook et al., 1971). Hypertrophied lenticels increase transport of oxygen to roots to oxidize the rhizosphere, thus avoiding anaerobic conditions in the root zone (Hook and Stubbs, 1967; Hook et al., 1970a**;** Hook et al., 1971). The adventitious roots of swamp tupelo tolerate high concentrations of carbon dioxide and are capable of undergoing anaerobic respiration (Hook et al., 1970a; Hook et al. 1971). Although the species is

regarded as tolerant of flooding, responses of swamp tupelos to flooding can vary among local populations (Hook and Stubbs, 1967). In contrast, black gum responds adversely to both root-zone inundation and extreme water deficit (Nash and Graves, 1993).

How the growth of swamp tupelo responds to flooding has been documented previously (Hook et al., 1970b; Hook et al., 1971), but there are no data on the drought resistance of the species. Moreover, direct comparisons of swamp tupelo and black gum subjected to various root-zone moistures have not been reported. The habitat of swamp tupelo is normally wet, but soils that support the species can dry during extended periods of drought (Outcalt, 1990). Because bottomland species such as *Alnus maritima* (Marsh) Nutt. (seasisde alder), *Pinckneya pubens* Michx. (fevertree), *Magnolia virginiana* L. (sweetbay magnolia), and *Taxodium distichum* L. (bald cypress) exhibit drought resistance (Nash and Graves, 1993; Schrader et al., 2005; Stewart et al., 2007), and because of the variable soil moisture in habitats where swamp tupelo occurs, we hypothesized swamp tupelo would display a superior resistance to water stress compared to black gum. Support for this hypothesis could result promote interest in swamp tupelo as a nursery crop, either as a specimen shade tree for managed landscapes or as a potential rootstock to enhance the water-stress resistance of black gum.

Our objective was to evaluate photosynthesis, stem water potential, and accumulation of biomass of seedlings of swamp tupelo subjected to water deficit and flooding; seedlings of black gum were used as a comparison. The root zones of both species were allowed to dry to different extents before rehydration and were maintained with differing extents of inundation. The data we present are the first to represent how swamp tupelo responds to a wide range of root-zone moisture contents and are the first direct comparison of stress resistance of these species.

Materials and Methods

Plant material. Fruits of swamp tupelo were collected in Oct. and Nov., 2013 from trees in Lake Prince, and Gates Co., NC. Fruits of black gum were collected from a cultivated tree at the Minnesota Landscape Arboretum, Chanhassen, MN and from a cultivated tree at Illinois State University, Normal, IL. Fruits were stored in polyethylene bags in a dark cooler at 5 °C before depulping and then stored in a cooler until initiation of cold, moist stratification. Seeds were cold-stratified for two months and germinated in March 2014. Individual seedlings were transplanted into plastic containers with heights of 14.6 cm, top diameters of 15.2 cm, and bottom diameters of 10.5 cm that were filled with a soilless, peat-based medium (Metro-Mix 900®; Sun Gro Horticulture, Agawan, MA, U.S.). Plants were fertilized twice weekly with Hi-Acid 28-18-8^{PLUS} fertilizer (Nutriculture® Spoon-Feeding® Soluble Fertilizer; Plant Marvel Laboratories Inc., Chicago Heights, IL, U.S.) at 150 ppm N in deionized H_2O until treatment initiation. On 9 June, 2 g of micronutrients (Micro Max®; Scotts Co., U.K.) were applied to the surface of the medium in each container. On 12 June, a foliar drench of iron chelate (Seqestrene® 138 Fe; BASF Corporation, Research Triangle Park, NC, U.S.) was applied at 10 g per 3.8 L of H_20 with a low-pressure sprayer to alleviate chlorosis.

On 24 June, plants were fertilized and allowed to drain for 2 h before initiation of treatments. Forty plants of each species were selected for uniform size and health. Four plants of each species, two from each seed source, were then randomly harvested for initial measurements of dry weight of plants. Initial heights (length of central leader from cotyledon scar to youngest node) of all plants were measured preceding the initiation of treatments and ranged from 26 to 48.5 cm and 9 to 20.5 cm for swamp tupelo and black gum, respectively.

Treatments. Seedlings were randomly assigned to one of six treatments of water regimes and positioned in a greenhouse in a completely randomized design, with six single-plant replications of each

species/treatment combination. Treatments consisted of three flood treatments and three drought-cycle treatments. Each container was placed in a plastic saucer to retain water for plants in flood treatments and to capture leachate from plants subjected to drought cycles. Plants treated with drought cycles were irrigated with 600, 500, or 400 ml of acidified tap water at a pH between 5.3 and 5.7 when moisture in the root zone decreased to below 5% (severe drought), 10% (moderate drought), or 20% (slight drought), respectively, and were allowed to sit in leachate for 30, 20, and 10 min, respectively. The progression of drought cycles was assessed for each plant individually, not on averages of replicates within treatments. After the first cycle was terminated for all plants in the severe-drought treatment by irrigation with 600 ml of acidified water, subsequent cycles of severe drought were ended by irrigations when root-zone moisture content was below 3%. Root zones of plants in the three flood treatments were either inundated with acidified water to 3 cm above the surface of the medium (complete inundation), or were saturated with water only in the lower 7.5 cm (wet) or 3.5 cm (moist) of the root zone. Measurements of root-zone moisture contents were taken by inserting an HH1 Theta Meter (Delta-T Devices, Cambridge, U.K.) into the upper 6 cm of the medium. On average, plants treated with severe, moderate, and slight drought completed two, five, and nine cycles during the experiment, which was performed with natural day lengths in a greenhouse in Ames, IA, with treatment imposition from 24 June and ending on 2 Sept. Air temperatures during the experiment ranged from 21 to 33 °C, relative humidity was 40 to 93.2%, and photosynthetically active radiation (*PAR*) was ≤398 µmol m^{-2} s^{-1}.

Collection of data. Every 14 d during treatments, photosynthesis was measured at the widest section of the blade of the youngest fully expanded leaf of each plant using a LI-6400 photosynthesis system (LI-COR, Lincoln, NE, U.S.) with a reference CO_2 of 400 µL·L^{-1} and 500 µmol·m^{-2}·s^{-1} *PAR*. The relationship between root-zone moisture content and photosynthesis was determined by measuring moisture content immediately before measurements of photosynthesis by using the HH1 Theta Meter.

Midday water potential of the xylem of the primary stem adjacent to the youngest fully expanded leaf of each plant was measured with a pressure chamber (PMS Instrument Co., Albany, OR, U.S.) immediately after root-zone moisture and photosynthesis were measured on the final day of treatments. Plants were harvested and surface area of leaf blades was measured with a LI-COR 3100 leaf area meter (LI-COR). The youngest fully expanded leaf, the oldest leaf, and the leaf midway between those two leaves were scanned after harvest for colorimetric analysis. Scanned images were uploaded to a computer and imported into winSEEDLE™ (winSEEDLE 2013 Régent Instruments Canada Inc.). A range of red and green color standards were assigned to determine the ratio of "red"-to-"green" pixels for each leaf (Burns and Dalen, 2002). An average was calculated for the three leaves of each plant. Leaves, roots, and stems were dried at 67 °C for at least 3 d and weighed. The formula of (Rate of dry mass accumulation) = (final dry weight) – (initial dry weight) / (number of days) was used to calculate the rate of dry mass accumulation of each plant. The formula of (increase in height) = (final height) – (initial height) was used to calculate the increase in height of individual plants.

Data analysis. All data were analyzed by analysis of variance and Tukey's honestly significant difference option of JMP® 11 software (JMP®, Version 11. SAS Institute Inc., Cary, NC, U.S. 1989-2007). Regression functions to describe the relationship of root-zone moisture content and photosynthesis were generated with SigmaPlot version 10.0 (Systat Software, San Jose, CA, U.S.).

Results

Plant survival. All plants of swamp tupelo survived the drought-cycle and flooding treatments. Leaves of swamp tupelos at the oldest two to four nodes became red and abscised without wilting when root-zone moistures were below 0.05 m^3 m^{-3}. Margins of youngest leaves of swamp tupelos became red after six weeks of treatments of moist and wet flooding. One plant of black gum died in the severe-drought treatment after its leaves wilted and abscised. All plants of black gum survived in the moist

treatment, one plant died in the wet treatment, and four plants in the complete inundation treatment died. Leaves of plants of black gum wilted during severe drought but regained turgidity after irrigation. Signs of stress from flooding among plants of black gum were red leaf margins, followed by leaf wilting and necrosis of leaves and stems. Some plants abscised leaves immediately after leaf wilting, while others had necrotic leaves persist on plants. Plants of black gum died approximately one week after symptoms of stress appeared. Only two plants of black gum in the inundated treatment were alive at the end of treatments, but most leaves of those plants were red.

Effects of drought on photosynthesis. Regression analysis showed that rates of photosynthesis of swamp tupelos were higher than those of black gums in the drought treatments during week two (Fig. 1A). Rates of photosynthesis of swamp tupelo and black gum were not different during weeks four to 10, therefore, data were combined (Fig. 1B). Within drought treatments, photosynthesis of swamp tupelo and black gum was not different across all weeks (Table 1).

Effects of flooding on photosynthesis. Rates of photosynthesis of swamp tupelos in the moist treatment were higher than those of black gums during weeks two and four but were similar thereafter (Fig. 2). In the wet treatment, plants of swamp tupelo maintained higher rates of photosynthesis than black gum from weeks two to eight but were similar during week 10 (Fig. 2). When completely inundated, plants of swamp tupelo maintained higher rates of photosynthesis than did plants of black gum (Fig. 2). The photosynthetic rate of swamp tupelos and black gums across all weeks were similar in the moist treatment, but as the degree of flooding increased, the photosynthetic rate of swamp tupelo surpassed that of black gum (Table 1). The photosynthetic rate of swamp tupelos across all weeks was 1.7 and 3.4 times higher than that of black gums in the wet and complete inundation treatments, respectively (Table 1).

Comparison of species. Across all treatments, the two species had similar stem water potential, and leaf area (Table 1). The dry weight of the roots of swamp tupelo was 1.6 times that of black gum (Table 1). Dry weight of shoots of swamp tupelo was 1.8 times that of black gum (Table 1). The total dry weight of swamp tupelo was 1.6 times that of black gum (Table 1). Rate of dry mass accumulation of swamp tupelo was twice that of black gum (Table 1), the swamp tupelos increased in height 7 cm more than did black gums on average (Table 1).

Effect of treatment on species. For swamp tupelo, severe drought led to the most negative stem water potential, whereas water potential of black gum was similar across treatments (Table 1). The leaf area of plants of swamp tupelo and black gum were similar within treatments (Table 1). Dry weight of roots of swamp tupelo was more than twice that of black gum after the moist treatment, but root weight of the two species was otherwise similar (Table 1). Dry weights of shoots of swamp tupelo were higher than those of black gum in all treatments of flooding, but similar in all treatments of drought (Table 1). The total dry weight of swamp tupelo was 2.2 times that of black gum after the wet treatment but was similar after all other treatments (Table 1). Among plants in the moist and wet treatments, rate of dry mass accumulation was higher for swamp tupelo than for black gum (Table 1). Increase in height of the two species was similar in moist treatments, but was four times greater for swamp tupelos than for black gums in the wet treatment (Table 1). The ratio of red-to-green stress index of leaves of black gums after treatments of complete inundation was higher than that of swamp tupelos, but similar in all other treatments (Fig. 4).

Discussion

Seedlings of swamp tupelo are capable of maintaining photosynthesis and surviving in root zones with a wide range of moisture contents. Swamp tupelo accumulated more total biomass, grew faster, and elongated primary stems more than did black gum across both drought and flooding (Table

1). Rates of photosynthesis of swamp tupelo and black gum were similar during drought across all weeks of the experiment, but swamp tupelo displayed a superior tolerance to flooding. Thus, we conclude that, because of its comparatively strong resistance to inundation, swamp tupelo should be evaluated further for its potential as a nursery crop that could be marketed as a shade tree that can withstand the extreme soil-moisture conditions common in urban and other managed landscapes.

All response variables were similar between the species within drought treatments. Additionally, swamp tupelo maintained greater rates of photosynthesis during week two, suggesting that swamp tupelo is better adapted than black gum to brief droughts (Fig. 1A). Swamp tupelo is exposed to periods of drought in the wild (Outcalt, 1990), which may account for its capacity to tolerate conditions of water deficit. Others (Nash and Graves, 1993; Schrader et al., 2005; Stewart et al., 2007) have found woody plants indigenous to wet to flooded habitats to be highly drought resistant.

Visual symptoms of stress periods of drought and flooding should be considered when evaluating a plant to be used in managed landscapes. The treatment of severe drought was changed to the threshold root-zone moisture content 0.03 m^3 m^{-3} because there was no visible stress on plants of swamp tupelo during the first round of drought cycles. Even when root-zone moisture contents dropped below 0.03 m^3 m^{-3}, wilting of leaves of swamp tupelo rarely occurred. One plant of swamp tupelo in the severe drought treatment displayed no wilting when root-zone moisture content was 0.01 m^3 m^{-3}, thus, certain genotypes of swamp tupelo may be more resistant to drought stress than others. When decreases in root-zone moisture evoked visible symptoms among plants of swamp tupelo, slight discoloration of the oldest leaves was followed by abscission. Leaf senescence and leaf abscission are responses of plants to water deficit and can contribute to plant survival (Munné-Bosch and Alegre, 2004). Discoloration of leaves was similar to the fall color displayed by the species and could have been due to production of anthocyanins and/or degradation of chlorophyll (Zwack and Graves, 1999). Using the ratio of red-to-green stress index of leaves as a method to quantify plant stress. We found that

black gum was more stressed than swamp tupelo when root zones were inundated, supporting results from Keeley and Franz's (1979) experiment (Fig. 4). All leaves of all plants of black gum with root-zone moisture contents below 0.05 m^3m^{-3} wilted but regained turgidity after irrigation. The capacity of swamp tupelo to avoid leaf wilting and maintain stem extension, and the abscission of only its oldest leaves at only extremely low root-zone moisture contents, suggest swamp tupelo may be better suited for withstanding drought than black gum.

Consistent with other studies (Hook et al., 1970b; Hook et al., 1971) we found swamp tupelo extremely tolerant of flooding. As the intensity of flooding increased, swamp tupelo maintained rates of photosynthesis that surpassed those of black gum (Fig. 2). Keeley and Franz (1979) also described what they considered the bottomland variety of black gum (*N. sylvatica* Marsh var. *biflora* (Walt.) Sarg.) to display a superior tolerance to flooding compared to the upland variety (*N. sylvatica* var. *sylvatica* Marsh.). Although swamp tupelo can photosynthesize at a greater rate than black gum under conditions of flooding, photosynthesis tended to decrease over time in all flooding treatments (Fig. 2). This could be attributed to seasonal changes or to limited availability of nutrients because plants were fertilized only at treatment initiation. As others have observed (Hook et. al, 1970a; Keeley and Franz, 1979), we noted the formation of adventitious roots and hypertrophied lenticels on submerged portions of the stems of swamp tupelo (Fig. 4). Keeley and Franz (1979) concluded that the adaptation to flooding of swamp tupelo is a sequential process with changes in anatomy and metabolism. They found that initial root systems of swamp tupelo began to deteriorate after one month of flooding and were replaced by roots capable of accelerated rates of anaerobic respiration. Between one month and one year of flooding, this new root system was replaced by a different root system that was morphologically distinct and resembled that of plants in well-drained treatments. These roots were capable of avoiding anoxia by increasing the internal transport of oxygen in the plant due to a breakdown of cortical tissue in roots. Hook et al. (1970a) found intercellular spaces were abundant in the complementary tissues and

phellogen of hypertrophied lenticels on flooded plants of swamp tupelo. In our study, adventitious rooting and hypertrophied lenticels did not occur on any plants of swamp tupelo in the other flooded treatments. The adventitious roots did not form on plants of black gum; roots systems of black gum in the inundated treatment were necrotic at the end of the experiment. Other authors have described the formation of adventitious roots on plants subjected to flooding (Gomes and Kozlowski, 1980; Pezeshki et al., 1996; Peterson and Graves, 2013).

Although swamp tupelo is capable of higher rates of photosynthesis when soils are intensely flooded, both species responded similarly to shallowly flooded root zones after four weeks (Fig. 1). This is not surprising because the ranges of both species overlap in areas that are wet and rarely inundated, such as margins of streams, and edges of ponds. If the anatomy and physiology of root systems of swamp tupelo allow for resistance to water stress, it may be advantageous for propagators to graft selected cultivars of black gum onto seedling rootstock of swamp tupelo. Because our study provided results from containerized seedlings in a greenhouse, we acknowledge that our conclusions should be viewed as tentative; results may have differed if the study had been conducted under field conditions, with older plants, or by imposing treatments differently. Some species of plants tolerate conditions of drought by establishing a deep root system, a mechanism that is restricted when using containerized plants. Contrarily, other studies have shown that reliable interspecific differences detected in greenhouse studies can be corroborated by subsequent research in the field (Myers and Landsberg, 1989; Schrader and Graves, 2000). The use of stagnant water rather than flowing water to impose flood treatments also likely influenced our results (Harms, 1973). Since our results support our hypothesis that swamp tupelo is more resistant to water stress than black gum, future experiments could be conducted to determine the grafting compatibility of swamp tupelo and black gum.

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Table 1. Mean photosynthetic rate (across all weeks), stem water potential, leaf area, plant dry weight, rate of dry mass acumulation, and increase in height of containerized seedlings of swamp tupelo (*N. biflora*) and black gum (*N. sylvatica*) subjected to six treatments of root-zone moisture content. Treatments were imposed on 24 Jun. 2014 and ended on 2 Sep. 2014. There were 36 single-plant replicates and six single-plant replicates per species and species/treatment combination, respectively. Means represent plants that survived treatments.

	Dependent variable							
Species and Treatment	Photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$)	Stem Ψ (-MPa)	Leaf area (cm^2)	Dry wt (g) Roots	Shoots	Total	Rate of dry mass accumulation ($g\ d^{-1}$)	Increase in height (cm)
Species across treatments								
swamp tupelo	5.4 A[z]	0.5 A	429 A	6.2 A	6.5 A	12.7 A	0.2 A	23.3 A
black gum	4.1 B	0.4 A	454 A	4.1 B	3.6 B	7.8 B	0.1 B	16.2 B
Drought								
Severe								
swamp tupelo	3 de[y]	1.2 a	235 c	3.2 cd	3.3 de	6.5 de	0.1 de	14.6 bcd
black gum	3.4 cde	0.6 ab	336 bc	2.7 cd	2.4 e	5.3 de	0.1 de	11.5 bcd
Moderate								

Table 1 continued

swamp tupelo	5.6 ab	0.4 b	446 abc	6.8 ab	6.3 bcd	13 abc	0.2 abc	24 abcd
black gum	5.7 ab	0.5 b	522.1 abc	4.8 bcd	4.2 cde	9 bcde	0.1 bcde	19.9 abcd
Slight								
swamp tupelo	6.2 a	0.3 b	472 abc	6.6 ab	6.9 abc	13.5 ab	0.2 abc	31.1 a
black gum	5.1 abcd	0.3 b	711 a	6 bc	5.5 bcde	11.5 bcd	0.2 bcd	22.6 abc
Flooding								
Moist								
swamp tupelo	6.2 a	0.3 b	651 ab	9.2 a	9.5 a	18.8 a	0.2 a	25.9 abc
black gum	4.7 abcd	0.4 b	425 abc	4.1 bcd	3.4 de	7.5 cde	0.1 cde	20.7 abcd
Wet								
swamp tupelo	6.1 a	0.3 b	479 abc	6.7 ab	7.6 ab	14.3 ab	0.2 ab	30 ab
black gum	3.6 bcde	0.5 b	311 c	3.6 bcd	3 e	6.6 de	0.1 cde	7.4 d
Complete inundation								
swamp tupelo	5.5 abc	0.3 b	290 c	4.5 bcd	5.4 bcde	9.9 bcde	0.1 bcde	14.5 abcd
black gum	1.6 e	0.3 ab	164 c	0.6 d	1.2 e	1.8 e	0 e	3.3 cd

[z]Species means within each column followed by the same capital letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

Table 1 continued

[y]Species/treatment means within each column followed by the same lower-case letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.

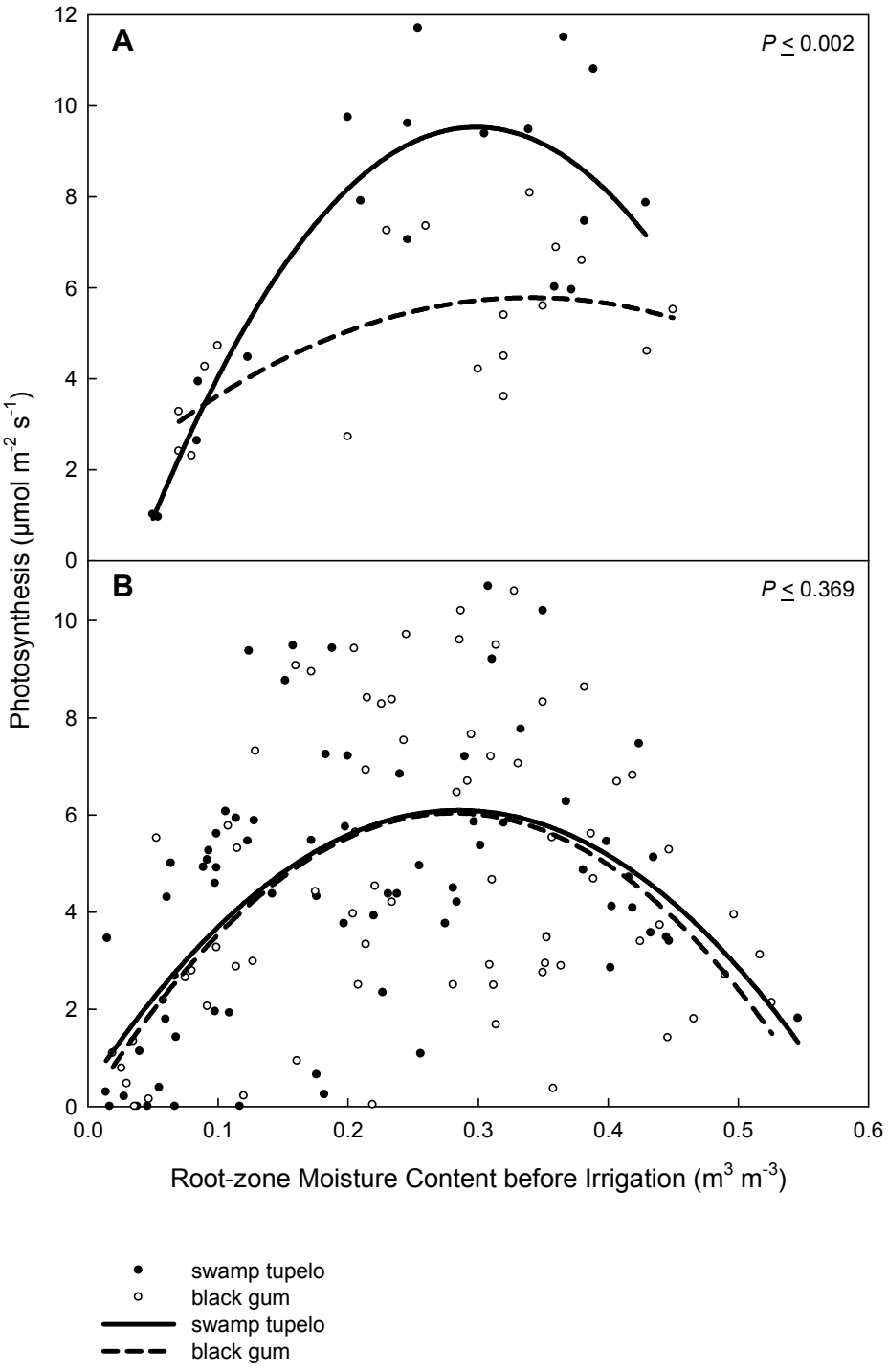
A
P ≤ 0.002
B
P ≤ 0.369
Photosynthesis (µmol m-2 s-1)
Root-zone Moisture Content before Irrigation (m3 m-3)
0
2
4
6
8
10
12
0.0
0.1
0.2
0.3
0.4
0.5
0.6
swamp tupelo
black gum
swamp tupelo
black gum

Fig. 1. Photosynthesis of swamp tupelo (*N. biflora*) and black gum (*N. sylvatica*) treated with drought cycles as a function of root-zone moisture. (**A**) Photosynthesis during week two (swamp tupelo: $y = -139.37x^2 + 83.19x - 2.8857$, $r^2 = 0.76$; black gum: $y = -37.273x^2 + 25.382x + 1.46$, $r^2 = 0.38$). (**B**) Photosynthesis measured every two weeks during weeks four to ten of the experiment (swamp tupelo: $y = -70.172x^2 + 40.011x + 0.3956$, $r^2 = 0.31$; black gum: $y = -73.61x^2 + 41.862x - 0.0162$, $r^2 = 0.26$). Each circle represents a measure of a single plant. Data from weeks four to ten were combined because there were no differences between species. A $P \leq 0.05$ in the upper right corner of the figure indicates that photosynthesis is better represented by two lines than one according to a three degrees of freedom test.

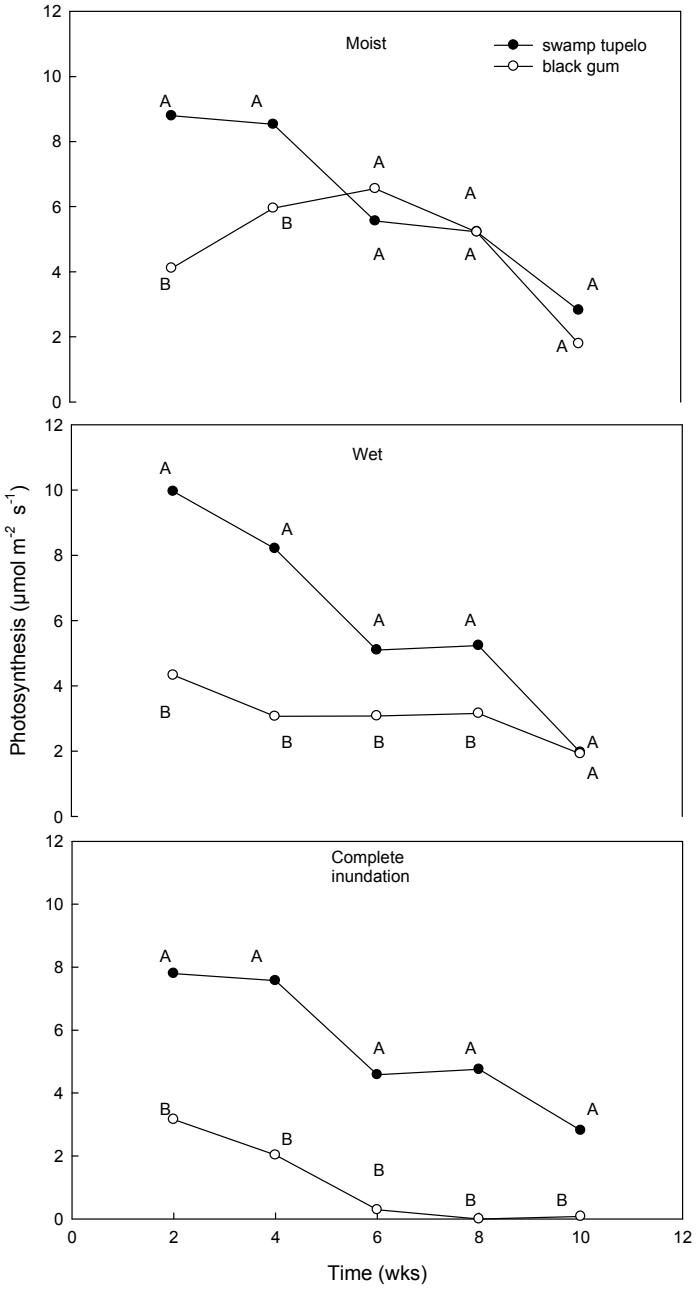
Moist
swamp tupelo
black gum
Wet
Complete
inundation
Photosynthesis (µmol m-2 s-1)
Time (wks)
0
2
4
6
8
10
12

Figure 2

Fig. 2. Photosynthesis of swamp tupelo (*N. biflora*) and black gum (*N. sylvatica)* across time (weeks) in each treatment of flooding (moist, wet, complete inundation). Different letters within a treatment and week indicate a significant difference in photosynthesis between species according to Tukey's honestly significant difference test at $P \leq 0.05$.

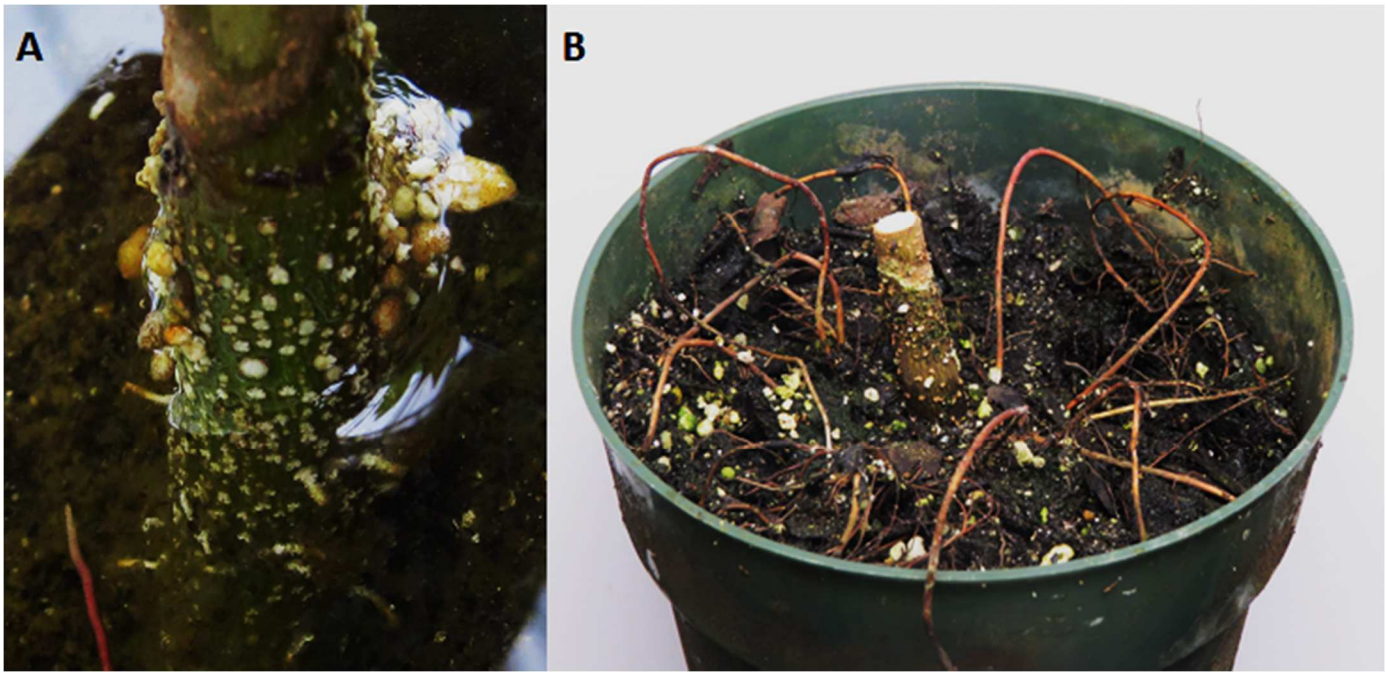

Fig. 3. (A) Proliferation of hypertrophied lenticels on a submerged and previously submerged portion of a stem of swamp tupelo (*N. biflora)* in the complete inundation treatment. Initials of adventitious roots are visible. (B) Adventitious roots developed near the surface of the water on all plants of swamp tupelo in the complete inundation treatment; neither phenomenon was evident on plants of black gum (*N. sylvatica).*

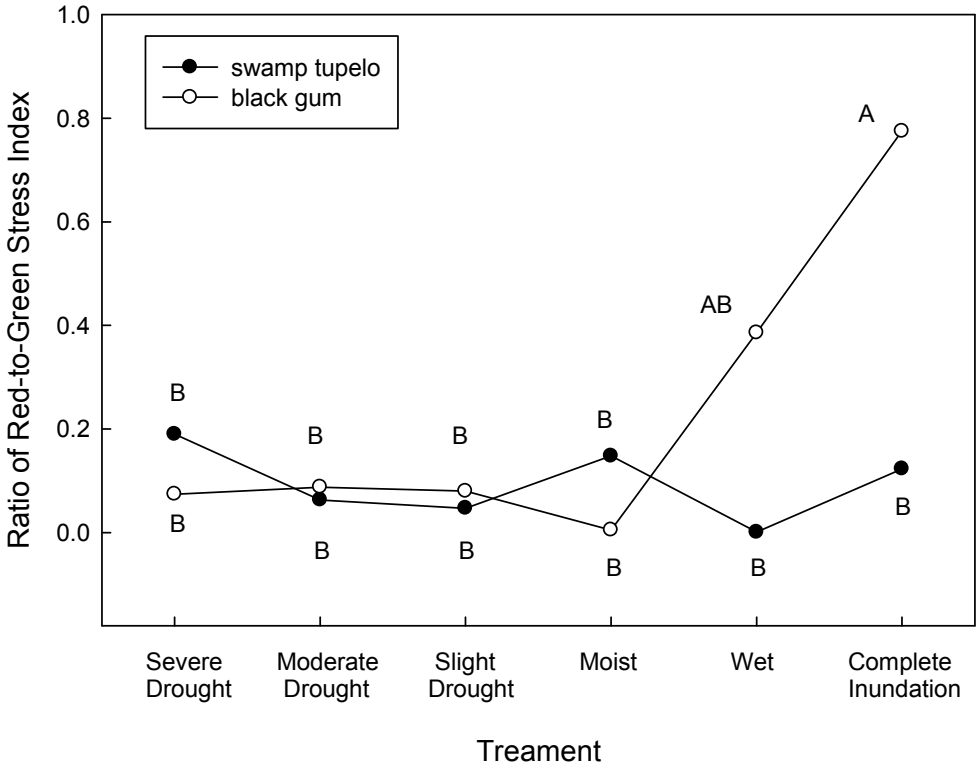

Fig. 4. Ratio of red-to-green stress index of swamp tupelo *(N. biflora)* and black gum *(N. sylvatica*) on the final day of the experiment. Different letters across species and treatments indicate a significant difference in ratio of red-to-green stress index according to Tukey's honestly significant difference test at $P \leq 0.05$.

CHAPTER 4. GENERAL CONCLUSIONS

Trees commonly known as ashes (*Fraxinus* spp.) make up a large component of the urban landscape due to their resistance to stress at disturbed sites. Green ash (*Fraxinus pennsylvanica* Marsh.) was one of the most commonly planted trees in urban areas throughout the Midwestern United States (Schoon, 1993). In recent years, the decimation of ashes by the emerald ash borer (*Agrilus planipennis* Fairmaire) in portions of the United States creates the need to identify alternative taxa that can withstand various urban stressors. Both drought and flooding are common in urban soils and may result in the decline of trees in cities (Whitlow and Bassuk, 1987; Berrang et al., 1985). Infiltration of water into the root zones of trees can be limited by compacted and crusted soils. Additionally, temperatures of urban soils can be higher than soil of natural areas, increasing the rate of evaporation of soil moisture (Graves and Dana, 1987).

Plants with capacities to resist stress from both drought and flooding are useful in urban plantings. Researchers have found plants indigenous to wetlands often display a resistance to drought stress despite the high water contents of soils of their natural habitats. Aware of this trend, we hypothesized that the hydrophyte, *N. biflora*, could have adaptions to drought. Although much is known about the tolerance of flooding of *N. biflora*, there have not been any studies conducted to evaluate the resistance to drought stress of *N. biflora*. Additionally, no studies have compared the responses of *N. biflora* to its congener, *N. sylvatica*, across a wide range of root-zone moisture contents.

In accordance with the trend of drought resistance in wetland taxa, we found seedlings *N. biflora* were able to survive in a wide range of soil moisture contents. Results from our experiment showed us that *N. biflora* may be more adapted to short-term periods of drought than is *N. sylvatica*. Additionally, we concluded that tolerance of flooding of *N. biflora* is superior to that of *N. sylvatica*.

Flood tolerance of *N. biflora* coincided with development of adventitious roots and hypertrophied lenticels on submerged stems. The long-term survival of *N. biflora* growing in anaerobic soils appears to be related to aeration of plant tissues.

Many seedlings of *N. biflora* will need to be evaluated before selecting a genotype with superior ornamental or physiological traits. We found that *N. biflora* can be propagated from seed with little technical difficulty. The pulp of fruits of *N. biflora* appears to inhibit germination, therefore, we recommend propagators remove pulp before sowing to enhance germination of seeds of *N. biflora*. Cold, moist stratification is a pretreatment that releases embryo dormancies of seeds. We found that total germination of seeds of *N. biflora* is not affected by time of stratification, but the vigorousness of germination on the peak day can be enhanced with 112 days of stratification at 5° C.

Future Research

Although my research provides basic insight on the horticultural potential of *N. biflora*, there are still other questions about *N. biflora* that could be investigated in the future. Because the natural distribution of *N. biflora* is restricted to the southeastern U.S., cold hardiness may limit the use of *N. biflora* in Midwestern landscapes. Cold hardiness studies in the lab and in the field can be conducted to test for effects of provenance on cold hardiness. It is possible that plants selected from northernmost portions of the range can be selected for cold hardiness traits. Because *N. biflora* produces consistent, red leaf color (Fig. 1 and Fig. 2), it is possible that male individuals could be selected for this trait.

Because we found seedlings of *N. biflora* to be more resistant to water stress than seedlings of *N. sylvatica*, it may be advantageous for propagators to graft readily marketed cultivars of *N. sylvatica* onto rootstock of *N. biflora*. Experiments can be designed to determine the best method for grafting *N. sylvatica* onto *N. biflora*; long-term success of grafts can then be evaluated. Once successful grafting protocols are established, it would be advantageous to subject grafted plants of *N. biflora* and *N.*

sylvatica to drought and flooding to determine if the use of *N. biflora* as a rootstock increases the resistance to water stress of scions of *N. sylvatica*. Although we found *N. biflora* and *N. sylvatica* similarly resistant to drought, it is possible the drought resistance of *N. biflora* may be altered after pre-exposure to flooding. Because *N. sylvatica* can be propagated from stem cuttings, one could attempt to propagate *N. biflora* from cuttings as well.

Interveinal chlorosis was observed on plants of *N. biflora* and *N. sylvatica* during culture in our greenhouse and may have been a result of the high pH (9.0) of tap water used for irrigation. Future experiments can be conducted to determine the effect of the pH of the growing media and water used for irrigation on health of plants of *N. biflora* and *N. sylvatica*. Due to a limited quantity of seeds of *N. sylvatica*, we did not investigate the effect of fruit pulp on germination of seeds of *N. sylvatica*, which may be of interest to investigate in the future.

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Fig. 1. Photograph of a specimen of *N. biflora* on the campus of Virgina Tech., Blacksburg, VA. Photo credit: R. Harris

Fig. 2. Photograph of leaves of *N. biflora* displaying fall color and mature drupes of *N. biflora* on the campus of Virginia Tech., Blacksburg, VA. Photo credit: R. Harris

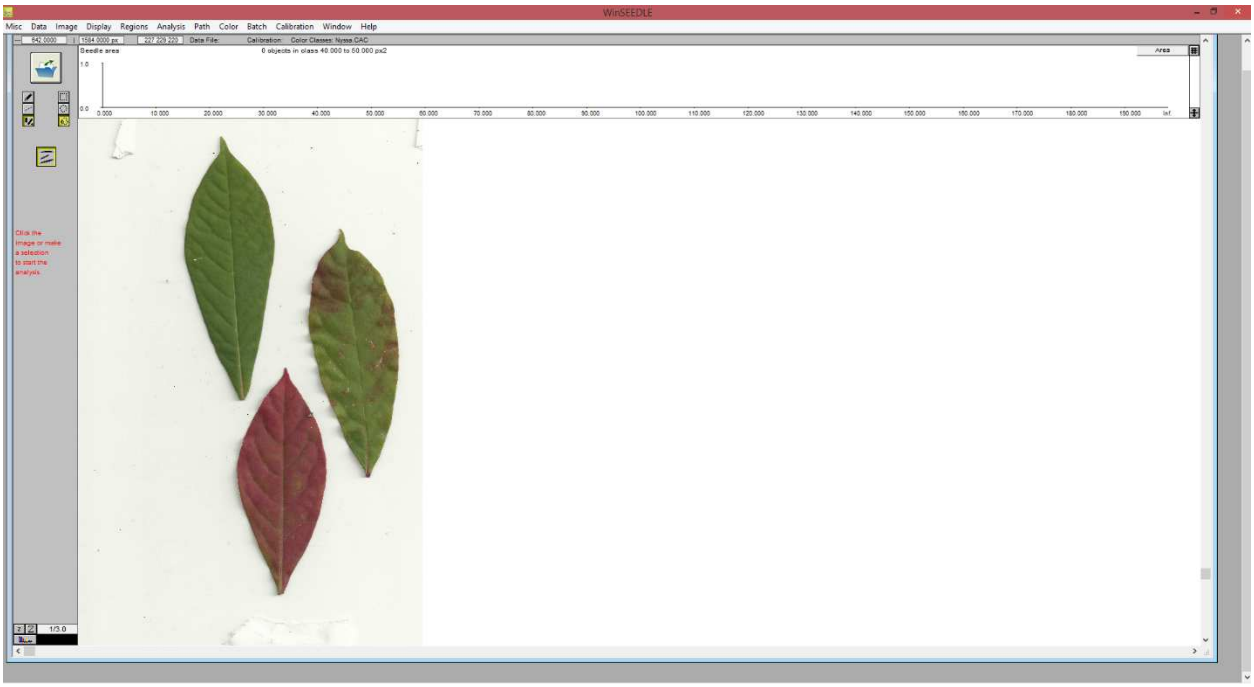
APPENDIX 1. PHOTOGRAPHS FROM WATER-STRESS EXPERIMENT

Adventitious Roots of *N. biflora*

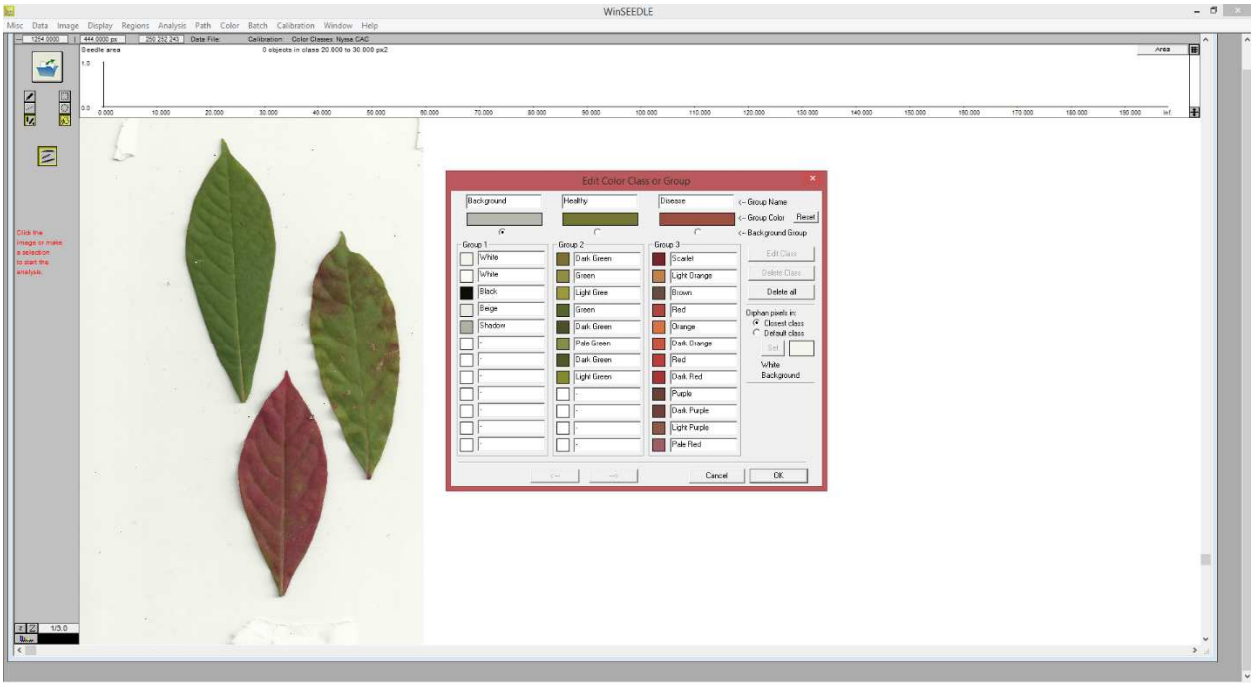
Hypertrophied Lenticels of *N. biflora*

APPENDIX 2. METHODOLOGY OF MEASURING RED TO GREEN STRESS INDEX OF LEAVES BY USING WINSEEDLE™ 2013

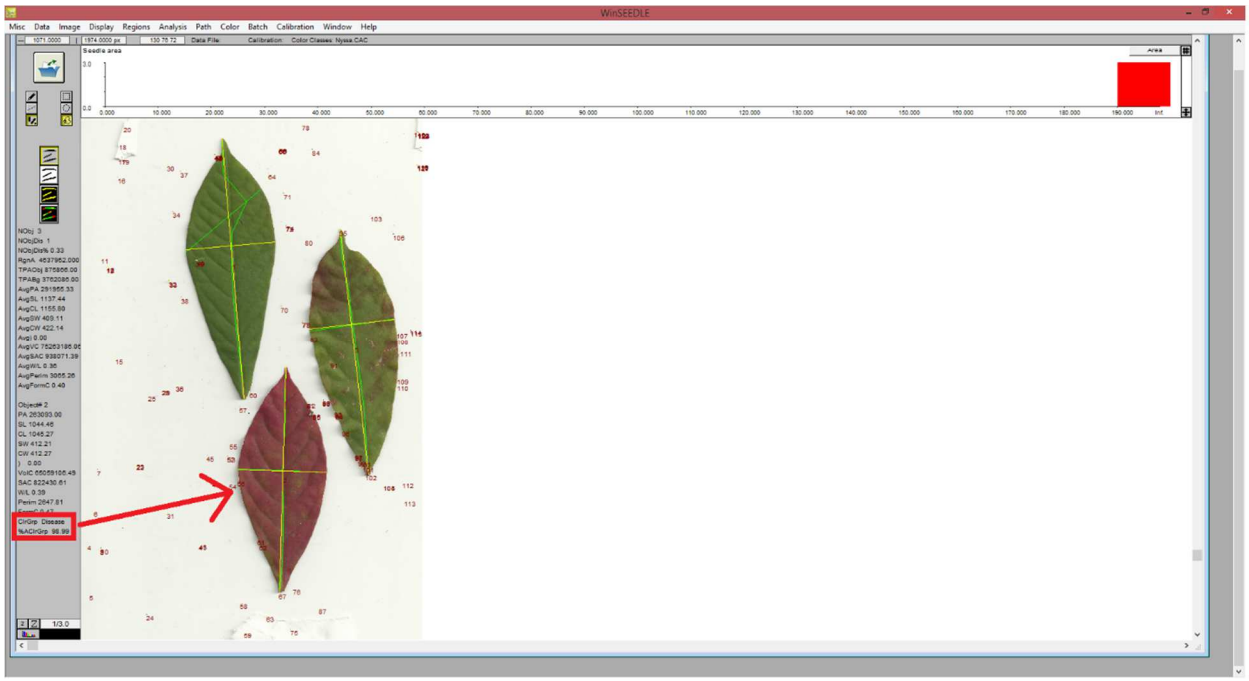
1) Upload scanned images of leaves



2) Define color classes for background area, healthy leaf area, and diseased (discolored) leaf area



3) Select a leaf to initiate color analysis; % area of leaf w/ discoloration will be displayed



APPENDIX 3. GRAFTING COMPATIBILITY OF *N. BIFLORA* AND *N. SYLVATICA*

Because results from our experiment support the idea that *N. biflora* is more resistance to water stress than *N. sylvatica*, we hypothesize the stress resistance of *N. sylvatica* can be enhanced by grafting *N. sylvatica* onto seedling rootstock of *N. biflora*. We conducted an experiment with the objectives of 1) determining the grafting compatibility of *N. bifora* and *N. sylvatica* and 2) determining the most successful method of grafting for the two species (bud insertion vs. whip graft). Seeds of *N. biflora* and *N. sylvatica* were collected from Hoke County, NC and the Minnesota Landscape Arboretum, Chanhassen, MN respectively, in fall 2013. Seeds of *N. biflora* and *N. sylvatica* were germinated in March 2014 and seedlings were transplanted into containers with top diameters of 17 cm, bottom diameters of 13 cm, and heights of 18 cm in May 2014. Plants were fertilized twice weekly throughout the summer in a greenhouse and were forced into dormancy by reducing air temperature in the greenhouse to 55° F in late November. Scions from a mature tree of *N. sylvatica* on the campus of Iowa State University (S of Union Drive Community Center) were also included in the experiment. The bud insertion was completed by excising a bud with 1 cm of stem tissue on each side and inserting it into a 2-cm wound on the stem of the rootstock between the cotyledon scar and the youngest node of the plant. The whip graft was completed by excising a 5-7 cm scion from the apex of the central leader and attaching it to the root stock directly between the cotyledon scar and the youngest node of the stem. The scions from the mature tree of *N. sylvatica* were excised from lower branches. All possible combinations of scions and rootstocks were used: *N. sylvatica* on *N. sylvatica* (control), *N. biflora* on *N. biflora* (control), *N. sylvatica* (mature) on *N. biflora* (heterograft), *N. sylvatica* on *N. biflora* (heterograft), and *N. biflora* on *N. sylvatica* (heterograft). Graft unions were wrapped in Parafilm M® (American National Can™, Menasha, WI) immediately after joining the bud onto rootstock or scion onto the rootstock. Plants were placed on a greenhouse bench in a completely randomized design beginning on

24 January 2015 with exposure to natural day lengths. Plants were misted twice daily and irrigated when the medium was visually dry. Data were collected after 53 days and are presented in Table 1. Unions were considered successful if any bud break was visible. The presence of chlorotic leaves was also recorded. Photographs of successful grafts follow Table 1. We conclude that *N. biflora* can be used as a rootstock for *N. sylvatica,* but we understand that our experiment is a short-term assessment of graft compatibility. Although bud insertion and whip grafts were successful for the combination of *N. sylvatica* on *N. biflora*, we observed that new shoots of scions of whip grafts were healthier and more vigorous than those of bud insertions. Future experiments can be conducted to graft *N. sylvatica* onto *N. biflora* with different methods of grafting and then evaluating long-term survival of grafted plants. It may be advantageous to include cultivars of *N. sylvatica* commonly marketed in the nursery trade, such as *N. sylvatica* 'Wildfire' and *N. sylvatica* 'Hayman Red' Red Rage®. Future research may also subject grafted plants of *N. biflora* and *N. sylvatica* to water stress to determine if use *N. biflora* as a rootstock improves stress resistance of scions of *N. sylvatica*.

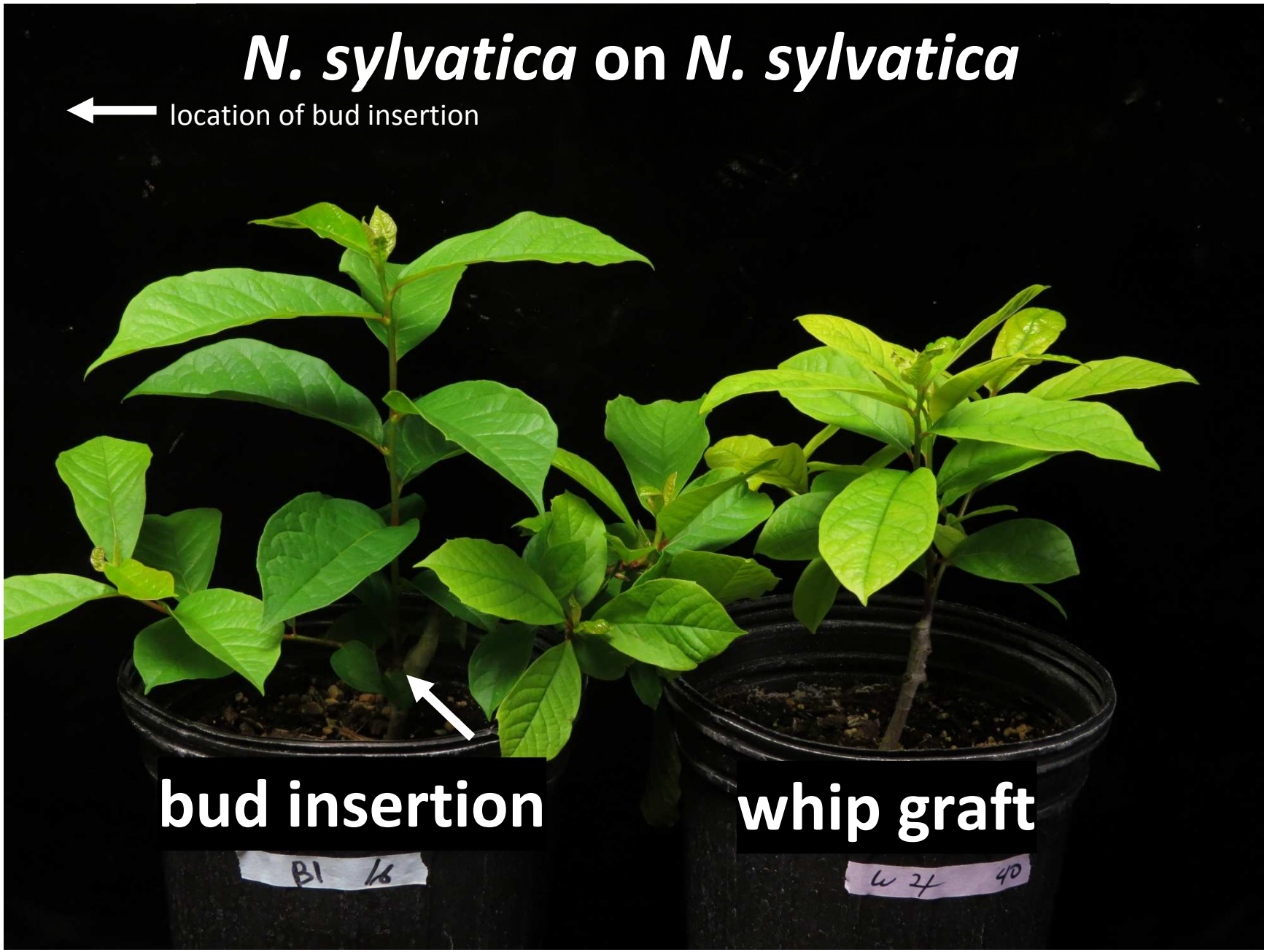
Table 1. Evaluation of budded and grafted plants of *N. biflora* and *N. sylvatica* 53 days after initiation of the experiment.

Species Combination and Method	n	Successful unions[z]	Successful unions w/ leaves displaying interveinal chlorosis	Average number of shoots per successful union	Average number of shoots ≥5 cm per successful union
N. sylvatica on *N. sylvatica*					
bud insertion	4	2	1	1.50	1.50
whip graft	4	4	1	5.75	1.00
N. biflora on *N. biflora*					
bud insertion	4	0	0	0.00	0.00
whip graft	4	1	0	4.00	2.00
N. sylvatica (mature) on *N. biflora*					
bud insertion	4	0	0	0.00	0.00
whip graft	4	0	0	0.00	0.00
N. sylvatica on *N. biflora*					
bud insertion	4	2	2	1.00	0.00

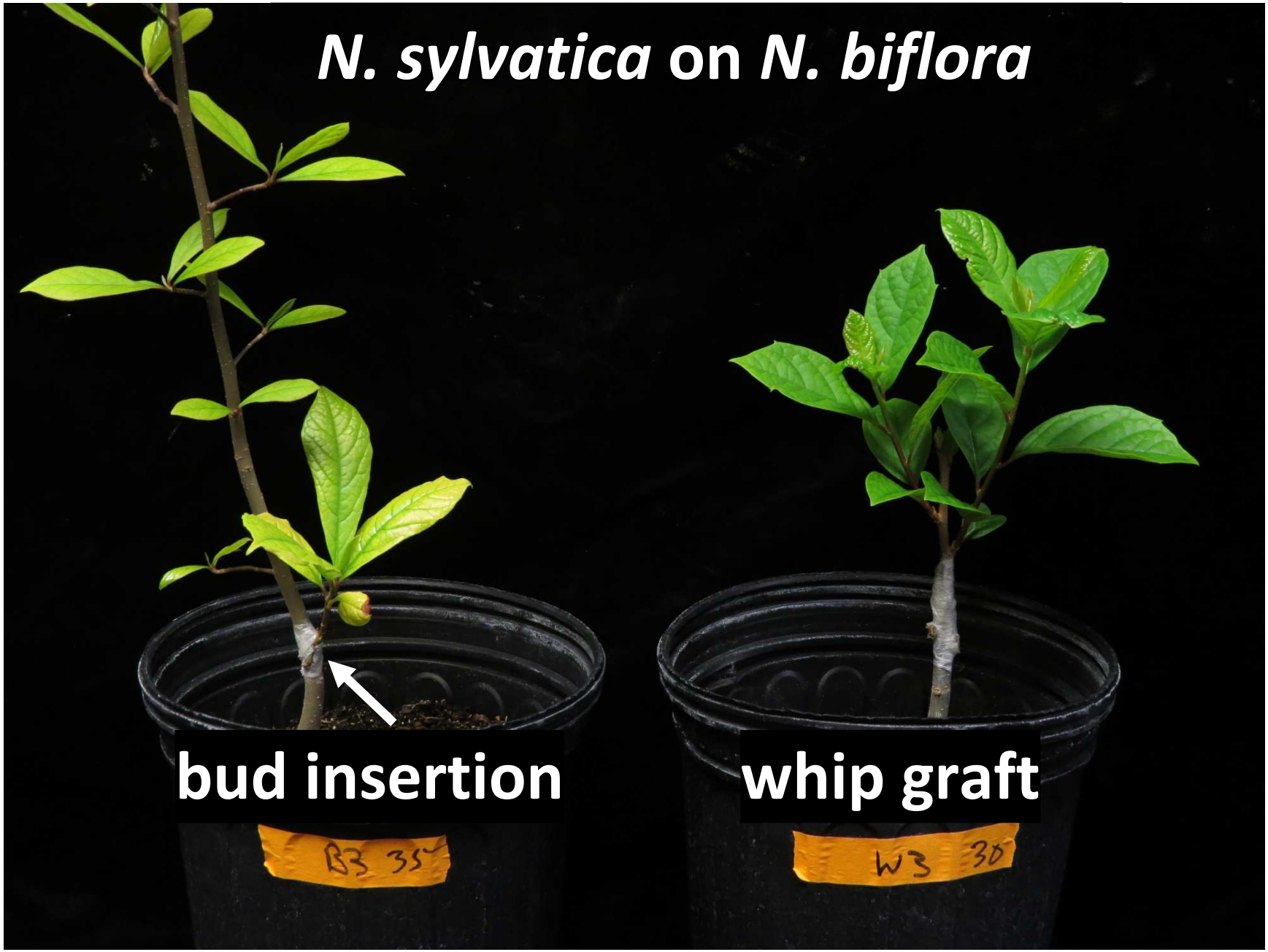
Table 1 continued

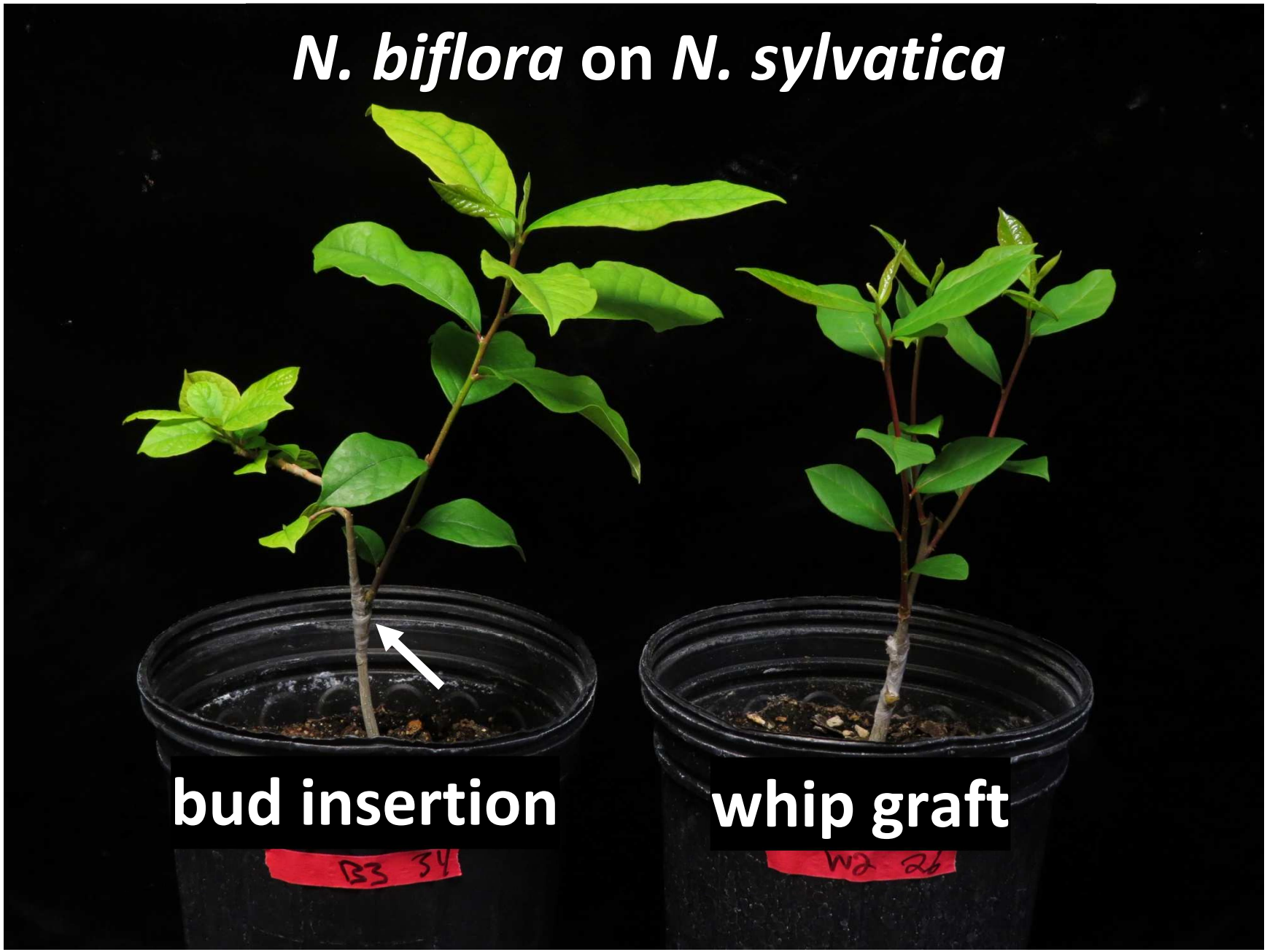
whip graft	4	4	1	2.25	1.25
N. biflora on *N. sylvatica*					
bud insertion	4	2	2	1.00	1.00
whip graft	4	2	0	2.00	1.50

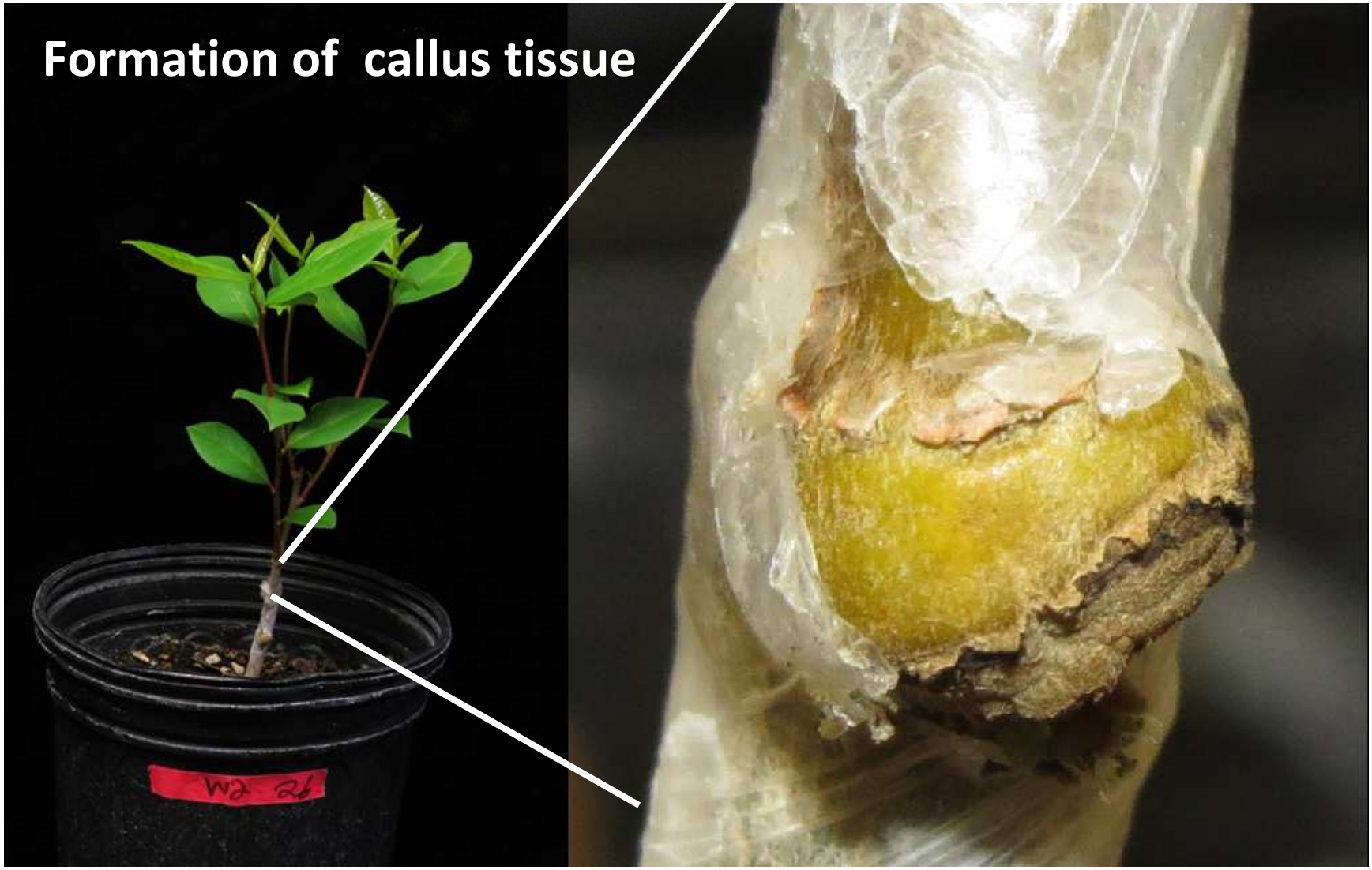
[z] Unions were considered successful if any bud break was visible.


N. sylvatica on N. sylvatica
location of bud insertion
bud insertion
whip graft

N. biflora on *N. biflora*


N. sylvatica on N. biflora
bud insertion
whip graft
B3 35
W3 30


N. biflora on N. sylvatica
bud insertion
whip graft


Formation of callus tissue

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